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IMMUNOHISTOPATHOLOGICAL STUDY OF *LISTERIA MONOCYTOGENES* INFECTION IN PREGNANT MICE

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Listeria monocytogenes is a gram-positive, facultative intracellular pathogen that can cause severe food-borne infections in humans and animals. Pregnant women, neonates, the elderly, and debilitated or immunocompromised patients in general are predominantly affected, although the disease can also develop in normal individuals. Using a murine model of pregnancy-associated listeriosis we have done a careful analysis of the host immune response to *L. monocytogenes* infection. We studied the kinetics of bacterial clearance, histopathological changes in maternal and fetal tissues, as well as cytokine/chemokine pattern during the course of *L. monocytogenes* infection.
Pregnant BALB/c mice challenged intravenously (i.v.) showed failure of maternal anti-listerial immune response at the systemic (significantly reduced serum IFN-g levels) and local level (reduced expression of proinflammatory cytokines and chemokines in the liver tissue), leading to devastating necrotizing hemorrhagic hepatitis. The insufficient maternal immune response also facilitated listerial multiplication in the placenta and, ultimately, in the fetus. Placental infection was characterized by large, hemorrhagic necrosis and numerous bacterial clusters. However, the inflammatory reaction in the placenta was confined to single granulocytes, while T-cells and other cellular elements necessary for effective antilisterial defence were absent. During the course of infection, TNF-a, and occasionally, IFN-g were transcribed in placental tissue. Increased placental levels of these anti-listerial cytokines were not sufficient to control bacterial growth, but may eventually contribute to spontaneous fetal loss and poor pregnancy outcome.

PCR BASED STRATEGIES FOR SUBGROUP-SPECIFIC CLONING OF MAP KINASE GENES FROM FILAMENTOUS FUNGI

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MAPKs of fungi are divided into three groups: YSAPKs (*y*east and fungal *s*tress *a*ctivated *p*rotein *k*inases), YERK1 and YERK2 (*y*east and fungal *e*xtracellular *r*egulated *k*inases). YERK1 and YERK2 MAPKs play an important role in the formation of pathogenesis related morphological structures, whereas members of the YSAPK subfamily are involved in stress adaptation processes. MAPKs are generally cloned by using PCR primers based on the protein kinase subdomains (I-XI) conserved in all MAPKs. The present work aimed at the development of a subgroup-specific approach, suitable for the selective cloning of YSAPK and other fungal MAPKs.
Based on multiple sequence alignment of more than 40 MAPK genes from 28 fungal species, we could identify 12 new hallmark sequences in the catalytic core, specific to the YSAPK MAPK subfamily. Only five motifs, I-b, IV-b, V-b, X-b1 and X-b2 showed specific patterns of amino acid residues for YSAPK MAPKs. Two of these motifs, I-b (SA[RK]DQLT) and IV-b (F[IL]SPLED[IV]) are absent in any other eukaryotic proteins. One of the YSAPK motifs, VII-a (IL[**VI**]**N**EN**CDL**) coincided with a loop spanning 7β-8β sheet present in human p38α (A**VN**ED**CEL**) and ERK2

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(**LN**TT**CD**L**K**, consensus in bold face) MAPKs. They may share a common function in interaction with L-X-L docking motifs of transcription factors.
Degenerate primers were designed for the above motifs and tested in a nested PCR assay. We could identify 526-991 bp long YSAPK MAPK sequence tags from eight filamentous species. All new sequence tags from these species, including *Fusarium culmorum, Fusarium proliferatum* and *Trichoderma harzianum* (NCBI GenBank accession numbers are DQ065608, DQ071424 and DQ071423, respectively) contained a TGY dual phosphorylation motif characteristic of YSAPK MAPKs. The right and left side flanking regions of the MAPK sequence tag cloned from *F. proliferatum* by using the YSAPK specific primers were then isolated by *s*ingle *o*ligonucleotide *n*ested (SON) PCR (Antal et al., Curr. Genet., 46, 240, 2004). This strategy utilizes two or more subsequent PCRs using only one sequence-specific nested primer for each run. Under low-stringency conditions, the same primer creates new specific annealing site(s) in bordering regions of the known sequence tag. Using this approach, the entire copy of a YSAPK MAPK gene, named *Fpmk3* was cloned from *F. proliferatum*. The catalytic core of the deduced FpMK3 protein showed 99.5 and 87.3 % identity with the corresponding regions of Osm1 and HOG1 YSAPK MAPKs from *Magnaporthe grisea* and *Saccharomyces cerevisiae*, respectively.
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EFFECTS OF COMBINED TREATMENTS OF MAP AND IRRADIATION ON ALFALFA SPROUTS

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Sprouts represent a specific issue because the sprouting procedure (conducted under high humidity at higher/elevated temperatures) is extremely favourable for the growth of bacterial pathogens. The behaviour of vegetable-associated pathogens after irradiation and MAP treatment will help to determine the applicability of these preservation treatments to these products as an antimicrobial intervention. Examination of the effect of irradiation on the survival of foodborne pathogens, such as *Listeria monocytogenes* and *Bacillus cereus* strains, inoculated on sprouts to improve the microbiological safety of these products has been carried out in the frame of an IAEA co-ordinated international project (11619/R0).
Two different gas mixtures were applied in MAP: 2% O_2, 4% CO_2, 96% N_2 (1) and 3-5% O_2, 10-15% CO_2 balanced with N_2 (2). Besides the effect of MAP, we examined the effect of the combination of low dose irradiation (1 and 2 kGy) and MAP on raw and inoculated alfalfa sprouts. Samples were stored at 5°C for 10 days. Microbiological and gas composition analysis were periodically carried out in triplicates. For the determination of radiation survivals, parallel with selective plating, Thin Agar Layer (TAL) method was performed to promote the recovery of the sublethally demaged cells. Because the traditional detection methods are labour- and time consuming, experiments were carried out to adapt a new, rapid impedimetric method for potential detection of *Listeria monocytogenes* on treated sprouts. Non-inoculated samples were used for sensory testing; after the combined treatment and at the end of the storage, panels of judges rated the samples on the basis of hedonic scores on colour, odour, taste and texture. Furthermore, another aim was to examine a possible relationship between the electronic nose results and irradiation doses.
The oxygen concentration was reduced to zero during storage at the 2nd (1) and 6th (2) days, respectively. Under the applied atmospheres, irradiation reduced the initial levels of the examined patho-

gens and that of the total microflora. During storage, *L. monocytogenes*, and mainly lactic acid bacteria from the natural microbiota regrew both on the irradiated and on the control samples. There were no differences between the selective plating and the TAL methods. The developed impedimetric method can be used to detect and enumerate *L. monocytogenes* present in numbers higher than 3 log CFU/g within 24 hours.
The sensory analysis showed no significant differences between treated samples. Results of electronic nose investigations indicated a significant difference between control (samples packed in air) and treated samples (MAP and MAP+ irradiation) at the day of the irradiation treatment.
Combination of low dose gamma-irradiation with modified atmosphere packaging and refrigerated storage can improve the microbiological safety and shelflife of alfalfa sprouts. Further investigations are necessary to develop the composition of head-space in MAP to be able to prevent regrowth of surviving pathogens during storage.

CONSPIRACY THEORY ON MAPK PATHWAY ELEMENTS AS INHIBITORS OF HIV-1 VPR PROTEIN

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HIV-1 is the most common type of the HIV viruses, and, similarly to SIV and other lentiviruses, a 96 -amino acid, 15 kDa virion associated protein Vpr is encoded by the viral genome. HIV-1 Vpr has been shown before to be responsible for G2/M cell cycle arrest which helps the viral replication cycle and enhances virus-associated pathology.
Induction of Vpr expression causes a number of cellular disfunctions including disruption in nuclear envelope structure, trans-activation of the viral promoter, and induction of apoptosis both in human and *fission yeast* cells. Some of these functions are thought to be caused by Vpr as an oxidative stressor though the pathway it is acting through is not yet clearly understood.
For the study of the linkage between oxidative stress and Vpr, *Schizosaccharomyces pombe,* a model organism was used with a single copy wt Vpr (NL4-3) integration in the genome. Here we show that upon H_2O_2 induction of different elements of the stress-activated mitogen-activated protein kinase cascade are able to interfere with some of the harmful effects of the Vpr protein, which supports our hypothesis that elements of the MAPK pathway are able to act as natural intracellular inhibitors of HIV-1 Vpr protein. Other strong evidence also suggests that the MAPK pathway is involved, though the exact molecular mechanisms still need to be identified.

MOVEMENT PROTEIN OF PLANT PATHOGENIC BYDV CAUSES MITOTIC ABNORMALITIES AND CELL CYCLE ARREST IN FISSION YEAST

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The barley yellow dwarf viral (BYDV) infection results in certain grain loss as a global disease in cereals. Like some other viral proteins the Movement Protein (MP) itself causes most of the symptoms during the BYDV infection both in plants and *fission yeast* model. MP expression contributes to hy-

per-phosphorilation of Cdc2 kinase resulting cell cycle arrest and retarded growth of the cultures. Unequal chromosome segregation and MP association with the nucleus were also shown. Our goal was to identify the target molecules and possible molecular pathways of this viral protein using genetic screening. During this project as Cdc2, Cdc25, Wee1 and PP2-A like phosphatase enzymes were found to be targeted by the Movement Protein, this means that the Movement Protein acts through a partially unknown cellular mechanism one part of it interacting with the G2/M checkpoint pathway. These results may suggest a new target and strategy for anti-viral treatments.

POLYMORPHISM OF MITOCHONDRIAL DNA AMONG *TRICHODERMA* STRAINS OBTAINED FROM MUSHROOM FARMS

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Trichoderma species are common soil-borne fungi, economically important because of their mycoparasitic ability, which makes them suitable for application as biocontrol agents against soil-borne plant-pathogenic fungi. Although *Trichoderma* species have been known as harmless microorganisms, green mold epidemics of commercially grown mushrooms caused by aggressive biotypes of *Trichoderma* species resulted in great economic losses both in Europe and in North America in the last two decades. The analysis of mitochondrial DNA, due to its relatively small size in comparison with the chromosomal DNA, is a useful tool in the comparative study of fungi. The aim of our experiments was to investigate the polymorphism of the mtDNA of *Trichoderma* strains obtained from Hungarian mushroom farms.

Trichoderma strains were isolated from *Agaricus* compost and *Pleurotus* substrate samples of three Hungarian mushroom farms, and the 20 strains with the best *in vitro* antagonistic abilities against *A. bisporus* were selected for detailed investigations. Total DNA was purified from the mycelia of the isolates and mtDNA RFLP patterns were obtained by digestion of the DNA samples with the *Hin*6I (G/CGC) restriction enzyme. Comparison of the mtDNA RFLP patterns with those of *Trichoderma aggressivum* f. *aggressivum* (CBS 450.95, CBS 100527 and CBS 100528) and *Trichoderma aggressivum* f. *europaeum* (CBS 100526 and CBS 433.95) strains obtained form the CBS culture collection revealed that four of the strains have the characteristic pattern of *T. aggressivum* f. *europaeum.* ITS sequence analysis confirmed that these four strains belonged to *T. aggressivum*. According to their ITS sequences, 11 isolates were identified as *T. harzianum*. Based on the mtDNA RFLP patterns, these isolates could be divided into four distinct groups, one of them containing isolates obtained from mushroom farms of different geographic locations. Three out of four *T. longibrachiatum* isolates shared a common pattern, which differed in three fragments (one restriction site) from that of the fourth isolate from another mushroom farm.

Examination of mtDNA RFLP patterns was used earlier to distinguish *Trichoderma* strains isolated from mushroom compost. In this study, we applied the method to investigate and compare the mtDNA RFLP patterns of *T. aggressivum* isolates with those of the type strains, and to examine the diversity of other compost-inhabiting *Trichoderma* species.

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DOUBLE-STRANDED RNA ELEMENTS IN *TRICHODERMA* STRAINS OBTAINED FROM MUSHROOM FARMS

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Trichoderma species are common fungi found in many cultivated and natural soils, and used as biological control agents of fungus-associated plant diseases. However, green mold epidemic of commercially grown mushrooms caused by *Trichoderma* species expanded in the last two decades both in Europe and in North America. Double-stranded RNA mycoviruses are found in the cytoplasm of numerous yeasts and filamentous fungal species. The infected fungi generally do not show any symptoms, but in certain cases special phenotypes are associated with their presence, like the killer features of *Saccharomyces cerevisiae* or hypovirulence in *Cryphonectria parasitica*. Although they are not infectious to intact cells and their propagation among individuals may occur only by hyphal anastomosis, some dsRNA viruses causing hypovirulence are used as biological control agents.
There is a lack of publications about the presence of dsRNA molecules in *Trichoderma* species. Earlier we have examined 105 *Trichoderma* isolates obtained from agricultural soil samples in Hungary and found three dsRNA-harboring strains amoung them, two *T. harzianum* and a *T. rossicum*. In this study, we investigated the presence of dsRNA molecules in green mold associated isolates obtained from Hungarian Agaricus compost and Pleurotus substrate samples, as well as from the CBS culture collection. All of the three *T. aggressivum* f. *aggressivum* strains obtained form the CBS collection contained three dsRNA elements (3.4, 4.3 and 10.0 kbp in size for CBS 450.95 and CBS 100527, and 2.4, 3.4, and 4.3 kbp in size for CBS 100528). Two out of the four *T. aggressivum* f. *europaeum* strains (CBS 100526 and CBS 433.95) contained two dsRNA elements of similar size (6.5 and 9.4 kbp), moreover, strain CBS 433.95 contained two additional elements of 3.0 and 10.0 kbp. In the case of the isolates obtained from Hungarian mushroom farms, all the four *Trichoderma aggressivum* strains contained the 6.5 and 9.4 kbp dsRNA molecules, moreover 2 of the 11 examined *T. harzianum* strains contained similar sized (6.5 and 9.4 kbp) elements, and a further *T. harzianum* isolate contained one 2.4 kbp dsRNA molecule. There were no dsRNA molecules found in the one *T. atroviride* and the four *T. longibrachiatum* strains examined.
In conclusion, it seems that the proportion of strains carrying dsRNA molecules is higher in the *Trichoderma* populations obtained from mushroom farms, than in those obtained from agricultural soils. It is well known that both *Agaricus bisporus* and *Pleurotus ostreatus* have different dsRNA viruses, therefore it can not be excluded that the *Trichoderma* strains obtained these molecules from the mushrooms during their coexistence in the compost. However, whether the dsRNA molecules have an effect on the virulence of the harboring strains is another interesting question.
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TAXONOMIC INVESTIGATIONS OF CLINICAL *TRICHODERMA LONGIBRACHIATUM* STRAINS

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Trichoderma species are filamentous fungi, commonly found in many cultivated and natural soils. Although *Trichoderma* species have been known as harmless microorganisms, certain members of this genus are emerging as causative agents of opportunistic fungal infections occurring with increasing frequency in the last two decades. An increasing number of common airborne and soil-borne organisms are continually being added to the list of potential fungal pathogens. Infections may occur when normal barriers of the human body are broken down (traumatic inoculation, surgery), host defenses are weakened by medical conditions or treatment, or in patients with chronic rhinosinusitis. The emerging incidence of these fungal pathogens is primarily the result of the increasing number of immunocompromised patients. From the genus *Trichoderma*, isolates belonging to the species *T. longibrachiatum* are most frequently reported to cause health problems in humans, ranging from localized infections to fatal, disseminated diseases.

To study the taxonomic relationships among *T. longibrachiatum* strains derived from clinical or soil samples, molecular characters were examined by the use of three molecular methods: restriction fragment length polymorphisms (RFLP) of the mitochondrial DNA, cellulose acetate electrophoresis (CAE) of isoenzymes, and sequence analysis of the internal transcribed spacer 1 (ITS 1) region. Molecular data were used to prepare dendrograms. The ITS 1 sequences of the strains do not contain enough variability to investigate the relationships among strains of the *T. longibrachiatum* species. CAE of seven enzyme systems (glucose-6-phosphate-dehydrogenase; glucose-6-phosphate-isomerase; 6-phosphogluconate-dehydrogenase; phosphoglucomutase; peptidases A, B, and C) was useful for dividing *T. longibrachiatum* strains into 6 electrophoretic types. Regarding the RFLP profiles of mitochondrial DNA, the examined isolates exhibited 7 and 10 different patterns using the restriction enzymes *Bsu*RI and *Hin*6I, respectively, and divided the strains into 4 groups on the dendrogram. These latter two methods produced more consistent profiles than the PCR-based RAPD technique and had a discriminatory power higher than that of the ITS sequence analysis.

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THE EFFECTS OF DEFOLIATION AND ACORN DEPRIVATION ON THE DEVELOPMENT OF OAK SEEDLINGS (*QUERCUS ROBUR* L.) AND THE MYCORRHIZAL LEVEL OF THEIR FINE ROOT SYSTEM: A FIELD STUDY

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Ecological mechanisms by which the biodiversity of plants and species composition are regulated and maintained are not well understood. We have very limited information about the belowground, hidden processes in the soil. Trees in the temperate and boreal zones almost exclusively live in symbiosis with ectomycorrhizal fungi. These fungi play a crucial role in the health of these forest trees by enhancing nutrient acquisition, drought tolerance, and pathogen resistance of their hosts. In return, the autotrophic hosts provide carbohydrates, vitamins, and hormones to their heterotrophic fungal partner. In this relationship, the two organisms have reciprocal influence on each others' life processes, but these interactions predominantly depend on the condition of the participants.

Here we study the influence of defoliation and/or acorn deprivation on the development and the mycorrhizal level of oak seedlings (*Quercus robur* L.), by the examination of 200 fine root tips per seedlings. The comparison of the short-time (6 weeks) and long-time (5 months) results indicate that the

mycorrhizal level on the roots was higher in autumn than in summer, and the two treatments have different impact on the survival rate and the mycorrhizal level of the oak seedlings. Deprivation of the acorns significantly decreased the growth and survival of the seedlings, whereas the mycorrhizal level of the fine root tips was unaffected. Deprivation of the acorns only reduced the mycorrhizal level of the fine roots when it was applied together with defoliation. Defoliated (up to two leaves) seedlings had significantly lower mycorrhizal levels on the root system, although their growth and the survival rates were unaffected.
The results suggest that diminution of the mycorrhizal level on fine roots of oak seedlings did not significantly affect the survival rate. On the other hand, the acorn deprivation, via different mechanisms, dramatically diminished the chance of the survival of the seedlings, and the comparatively high mycorrhizal level was not able to compensate this effect. Further morphological and molecular examination is needed to detect if there was any change in the fungal species composition on the mycorrhizal root tips. According to these results, it seems that beside the well known effect of edafic and microclimatic factors, the impact of acorn deteriorater and/or consumer organisms (e.g. rodents, pathogenic fungi, insects) on the fine scale spatial pattern of the oak seedlings in nature can be considerably higher than it was supposed previously.

TOWARDS AN EFFICIENT AND INTEGRATED BIOGAS TECHNOLOGY

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Most biogas production technologies in use today are based on the biological activity of microbial consortia developing spontaneously during the start-up phase of the anaerobic digestion process. This provides usually satisfactory results in spite of the obvious fact that these microbial consortia are not optimized for maximum biogas production. Biogas is the ultimate excrement of these consortia and the natural equilibrium prefers optimal growth rather than optimal biogas production. Among the significant recent advances in understanding the ecology of anaerobic biodegradation of organic wastes is the recognition of the close syntrophic relationship among the three distinct microbe populations and the importance of H_2 in process control. The regulatory roles of hydrogen levels and interspecies hydrogen transfer optimize the concerted action of the entire population. The concentration of either acetate, hydrogen, or both, can be reduced sufficiently to provide a favorable free-energy change for propionate oxidation. During anaerobic biodegradation, hydrogen concentration is reduced to a much lower level than that of acetate. In addition, the hydrogen partial pressure changes rapidly, varying by an order of magnitude or more within a few minutes. This is related to its rapid turnover rate. The energy available to the acetate-using methanogens is independent of hydrogen partial pressure, whereas, in the case of hydrogen-producing and hydrogen-consuming species, it is very much a function of it.
We have shown that under these circumstances addition of hydrogen producers to the system and thereby shifting the population balance brings about advantageous effects for the entire methanogenic cascade. The decomposition rate of the organic substrate increases and both the acetogenic and methanogenic activities are remarkably amplified. In laboratory experiments some 2.6-fold intensification of biogas productivity has been routinely observed and the same results were obtained in scale-up experiments. Proper management of the bacterial population is expected to facilitate the start-up of fermentation. In order to reduce the costs of this treatment, supplemented bacteria are grown in diluted industrial wastewater. In contrast to the commonly used factor of 0.6-0.8 that is used to estimate biogas yields, the integrated technology, using intensified microbiological biomass decomposition, should yield a two-three fold increase when using 15% solid content biomass. The integrated tech-

nology uses sugar accumulating plants (e.g., sweet sorghum, Jerusalem artichoke) to increase the biodegradable content of the substrate biomass.

PHYLOGENETIC ANALYSIS OF TAHYNA VIRUS STRAINS ISOLATED IN CENTRAL-EUROPE

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Tahyna virus is an arbovirus, which belongs to the California group of the genus Orthobunyavirus, within the family Bunyaviridae. It was first isolated in 1958 from mosquitoes in the Slovakian village of Tahyna. Besides wild and domesticated animals, the virus is able to infect humans as well. In patients observed so far, infection with Tahyna virus appears with influenza-like symptoms (fever and mild respiratory disease). In some cases, meningoencephalitis and atypical pneumonia were observed. It occurs in most countries of Central and Southern Europe, and is the most common in Central Europe. In Hungary, the virus was first isolated in 1967 from *Culiseta* (*Theobaldia*) *annulata* mosquitoes collected near by Murakeresztúr. The virus was also isolated several times in South-Moravia (Czech Republic) from mosquitoes and from ill children ("Valtice fever"). In 2004, the nucleic acid of the virus was detected in the peripheral blood mononuclear cells (PBMCs) of human patients from Lower Austria.
In this study, the S and M genome segments of Tahyna virus strains were amplified in RT-PCR assay, the products were sequenced and the sequences were submitted for phylogenetic analysis. The investigated strains from Slovakia, the Czech Republic, Austria and Hungary have shown high levels of sequence identity (99%) in the two genome segments coding for the main structural proteins N, G1 and G2. The results of the investigations indicate that the Tahyna virus is a relatively less variable virus; therefore, the strains circulating in Central-Europe have similar pathogenicity, and therefore potential human health impact. Molecular diagnostic methods could improve the efficacy of the detection of Tahyna virus in human patients in the acute phase of the infection.

GENETIC ENGINEERING OF TRANSGENIC VIRUS RESISTANT PLANTS – A SUCCESS STORY

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Since the foundation of the Agricultural Biotechnology Center (1990) at Gödöllő, where I launched a research program on plant genetic engineering for virus resistance, several engineered crops were introduced for field trials. Plant viruses were isolated from the Hungarian flora and their primary structures were identified. By exploring their structural organizations, their genes were cloned into plant expression vectors and their functions were studied in vitro and in vivo. Following the first report on obtaining virus resistant transgenic plants, we produced several economically important virus resis-

tant plants such as tobacco, potato and pepper. Potato virus Y resistant tobaccos were tested for ten years in small-scale field plots, and the transformed hybrids showed a high level of durable virus resistance. In the case of potato it was possible to reincarnate old Hungarian cultivars, those disappearing from cultivation due to their high sensitivity to the necrotic and dominant isolate of potato virus Y. Along with the production of virus resistant plants, several bio-safety concerns were carefully analyzed. Constant monitoring of the virus population has been performed, and recombinant viruses were not recorded in fields where different tobacco breeding lines were grown, including transgenic ones. Aspects of the potential ecological impacts of virus resistant plants were studied such as the possibility of heteroencapsidation, synergisms and gene flow. All of our research data are in a good agreement with the successful commercial planting of transgenic virus resistant papaya in the US.

EFFECT OF METALS ON THE MICROBIAL COMMUNITIES OF THE UPPER-TISZA AND SZAMOS RIVERS

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In the basin of the Upper-Tisza, there are 43 industrial by-product disposal sites, such as: mines, chemical stations, as well as pig farms releasing untreated communal waters that are actual or potential sources of surface water pollution. In the light of the almost sequential cyanide and the subsequent metal contaminations in 2000, the detailed examination of the biosphere seems to be more than reasonable. In 2000, nearly 100 000 m^3 water with a huge cyanide content (110–130 tons of cyanide) entered the River Tisza via the River Szamos. This water strongly contaminated with cyanide also landed enormous quantities of metals, e.g. 70–100 tons of copper and even larger amounts of zinc, into the Tisza, and the impacts of these metals were hardly highlighted at that time in the face of the havoc caused by the much more dangerous cyanides. In a few months, 20 000 tons of sludge contaminated with heavy metals reached the River Tisza. With respect to the foregoing, our aim was to examine the ecotoxicological impacts of various contaminants on the biosphere of the water and flood plains, the resistance of microorganisms to contaminants, as well as the mobilization, immobilization, accumulation and transformation of contaminants. Studies on the Upper-Tisza and its environs have been implemented at eight sampling sites in the river tract between Rahiv (the Ukraine) and Dombrád, as well as at the location where the River Szamos reaches the national border. Results: The pH of the Tisza as measured along the current line between Rahiv and Dombrád was 7.52–9.01. On the basis of similarities and differences in the microflora of the River Szamos, as well as that of the lower and upper stretch of the Tisza it can be ascertained that the microorganisms in the microflora of the River Tisza that shows tolerance to high metal contents originate from the tributaries of the river. Especially, it is the River Szamos that exercises considerable influence on the microbiological state of the Tisza. Bacterial and yeast organisms being typical of the Upper-Tisza, as grown in Nutrient medium*: Enterobacter agglomerans, Pseudomonas stutzei, Chryseomonas luteola, Vibrio metschnikovii, Xanthomonas maltophilia, Candida glabrata, Candida humicola, Candida inconspicua, Candida lambica, Cryptococcus laurentii, Rhodotorula minuta, Saccharomyces cerevisiae, Trichosporon cutaneum, Trichosporon pullulans,* their number ranges from 2.5×10^9 to 9.0×10^9 per cubic metre. Microorganisms surviving the 24-hour and 48-hour, 1000 mg/kg Cu, Ni, Cd, Zn and Pb ion load in the River Tisza and the River Szamos are: *Aeromonas hydrophila/caviae*, *Branhamella catarrhalis, Chryseomonas luteola, Enterobacter agglomerans, Flavobacterium indologenes, Flavobacterium meningosepticum, Klebsiella apiculata, Pseudomonas stutzeri, Pseudomonas chlororaphis, Pseudo-*

monas vesicularis, Psudomonas pseudomallei, Sphingomonas paucimobilis, Vibrio metschnikovii, Xanthomonas maltophilia bacteria, *Cryptococcus terreus, Candida glabrata, Candida humicola, Candida inconspicua, Candida lambica, Candida magnoliae, Kloeckera japonica, Rhodotorula minuta, Saccharomyces cerevisiae, Sphingomonas paucimobilis, Trichosporon capitatum, Trichosporon cutaneum, Trichosporon pullulans* yeasts.

PHYLOGENETIC ANALYSIS OF HUNGARIAN PRRSV STRAINS

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Porcine reproductive and respiratory syndrome (PRRS) is a widespread disease in swine, characterized by reproductive disorders in gilts and sows and by respiratory disorders in all ages, sometimes leading to death mostly in neonatal, suckling and weaned piglets. Marked genetic differences have been observed between the European and North American strains, moreover, there is different cluster among the European strains isolated in Lithuania, which is thought to be the common ancestor of the European and American strains. The most variable region of the genome of PRRSV is the ORF5, the coding region of the 25-kDa membrane associated glycoprotein, which most probably plays a role in the attachment to susceptible cells, since antibodies produced against this protein can inhibit infectivity. The aim of this study was to detect and characterize PRRSV strains demonstrated from porcine samples sent for investigation from different locations in Hungary, and to analyze their relation to other European isolates by comparing their ORF5 sequences.
The samples were sent for diagnostic investigation from large-scale industrialized farms experiencing economic losses due to respiratory disease or reproductive disorders. Sera obtained from live animals, or lung homogenates of piglets dying of respiratory syndrome, were tested and reacted positively in the RT-PCR using primers designed to amplify the most conservative region of the genome, detecting both the European and the American strains. The positive samples were subjected to another RT-PCR with different primers to amplify a region of, or the whole ORF5. For this reaction, we used two pairs of primers, one specific to the European and the other to the American strains.
Analyzing the data, we found that all Hungarian sequences belonged to the European genotype showing 90-95% nucleotide identity to each other. We could also isolate a strain from the alveolar macrophages of a clinically healthy piglet that had only one nucleotide (and amino acid) difference compared with a live vaccine virus.

TRIALS FOR VIRUS DETECTION AND ELIMINATION OF PEACH CULTIVARS UNDER IN-VITRO CONDITIONS

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The importance of healthy planting material is a continuous concern both in the EU and in Hungary. Economic losses caused by stone-fruit orchards highly infected by virus or phytoplasma reach considerable amounts. In vitro micropropagation is a method for quick production of uniform planting material as well as of certified elite plants of new cultivars. The recently developed molecular patho-

gen detection procedures for PPV, PDV, PNRSV and ESFY are very sensitive and thus require only a limited amount of plant material for the examinations. The combination of micropropagation, pathogen detection and elimination under in vitro conditions offers a good opportunity to increase the effectiveness of healthy planting material production. The growth habit of virus-infected plants is reduced and characterized by a high mortality rate even under in vitro condition. The development of a micropropagation procedure for virus-infected peach cultures was based on the multiplication method of healthy plantlets previously established in our lab. Single established shoot tips were tested by ELISA or IC – RT – PCR for PPV, PDV and PNRSV and by PCR for ESFY. Peach cultures are very sensitive for high temperature during thermotherapy. The suitable medium for growth under high temperature, the proper light intensity, temperature and longevity of the minimal required thermotherapy for achieving shoot-tips free of infections are under determination. The shoots growing from meristematic tips isolated right after finishing the heat-treatment are tested for pathogens as soon as possible. Only healthy shoots, free of any infections, will be cultured on, multiplied and raised for the growers under in vitro condition during the long period of bio-tests. Those shoots that appear infected by molecular tests are eliminated immediately, which is also a cost-saving method, since only the healthy shoots will be grown further, acclimatised to greenhouse conditions and integrated in the plant production process.

Acknowledgement: Supports by the BMBWK (Austria) project „Improved strategies for assuring the phytosanitary and genetic quality for stone fruit planting material" and by the GKM (Hungary) project „Virus elimination of stone fruit cultivars and virophyl ornamental plants under in vitro conditions" are kindly acknowledged.

EPIGENOTYPES OF EBER 1 AND 2 GENES OF EPSTEIN-BARR VIRUS IN LYMPHOID AND NASOPHARINGEAL CARCINOMA CELL LINES

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Epstein-Barr virus (EBV) encodes two protein-noncoding RNAs (EBER 1 and 2), which are constitutively expressed in latently infected host cells. Transient transfection and *in vitro* binding assays identified a series of regulatory elements in the RNA Polymerase III (Pol III) transcribed EBER promoters. In order to characterize the epigenetic mechanisms affecting EBER expression, we examined DNA methylation, histone acetylation and histone methylation patterns in EBER regulatory sequences. We also analysed the effect of *in vitro* DNA methylation on EBER 1 transcriptional activity. Using the method of bisulfite sequencing, we found extensive hypomethylation in the EBER region in six cell lines representing the three major EBV latency types. Using chromatin immunprecipitation, we showed that the level of histone H3 and H4 acetylation, and methylation of lysine 4 in histone H3 did not correlate with EBER 1 expression. We observed that *in vitro* DNA methylation of the EBER 1 gene resulted in an almost complete inhibition of its transcription in transfected B- and epithelial cell lines. These results indicate that the highly methylated epigenotype of Pol III transcribed EBER 1 and 2 genes is an important factor in maintaining their constitutive activity.

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PREDICTIVE MICROBIOLOGY TOOLS FOR QUANTITATIVE MICROBIAL RISK ASSESSMENT

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Predictive microbiology: Since the 1980's, quantitative approaches have become a "must" for food microbiology research projects. An outcome of this development is the increasing number of reference to a discipline called "predictive food microbiology". The name was coined in the 1930's but practical applications only began to be realised in the 1980's, with the everyday use of powerful desktop computing. Perhaps "quantitative microbial food ecology" would be the best description of the subject, but because of historical reasons we stick to the name as commonly used today.
Mathematical modelling, the core of predictive microbiology, is based on simplifications. For the so called *primary models*, the idealisation is that, in a constant environment, a homogeneous bacterial culture grows at the same specific rate. This *exponential phase* is preceded and followed by the *lag* and *stationary* phases. Introduction to predictive microbiology courses commonly start with defining the parameters of this sigmoid shape. Models describing the effect of environmental factors (mainly temperature, pH and water activity) on the most important growth parameters, notably on the specific growth rate, are called *secondary models*. In case of the secondary models, the idealisation is that it is enough to consider only a few environmental factors in order to characterise the responses. The practical potential of this approach is obvious; software packages based on the developed mathematical models could predict the growth of spoilage and pathogenic microorganisms as a function of the processing and storage environment of a certain food. Application to Quantitative Microbial Risk Assessment: Predictive models are useful tools to assist HACCP systems, product development and quantitative microbial risk assessment. The latter one requires estimating not only the kinetic parameters of bacteria but also their probability distributions. Concepts like variability and uncertainty, which are central in QMRA procedures, are still frequently misunderstood and misinterpreted in those studies. Quantification of variability is based on measurements that can be replicated under the same conditions. Therefore, large amounts of data are necessary to validate probabilistic models, implying the increasing effort to invest in the automation of measurements and the development of systematically organised databases for the collected data. On the other hand, uncertainty is related to the knowledge missing from the mathematical models behind the applied risk assessment framework. Thorough analysis of predictive microbiology models can help to use both concepts correctly.

EFFECTS OF AGROTECHNOLOGY, COLD STORAGE AND MAP ON THE EPIPHYTIC MICROFLORA OF STRAWBERRIES

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The epiphytic microflora of three strawberry varieties (Camarosa, Honeoye, Elsanta) cultivated in open fields was investigated immediately after harvest and during storage for 2 weeks at 1°C. Viable counts of total mesophilic aerobic, *Enterobacteriaceae*, *Salmonellae*, *Listeria monocytogenes*, *Bacillus cereus*, coliforms, *E. coli*, moulds and yeasts were determined on the fruits and in the soils. Weight loss was also measured. The soil with straw covering, and the strawberries grown on covered

soil were more contaminated than without the cover. Rainy weather during harvest might have had an influence on microbial contamination.
For cv. Elsanta modified atmosphere packaging (MAP) was carried out (O_2 2 %, CO_2 4 %, N_2 94 %), and samples were stored at 1°C. The changes in gas composition were measured during storage. Strawberries without packaging dried slightly, but the flavour was still not damaged at the end of storage (14 days). MAP prevented the drying of fruits, and though the microbial counts have not changed, at day 8 the strawberries were fermented due to the moisture and anaerobic conditions.
Honeoye cultivar grown under identical conditions to the ones before was also investigated in the following year, and the results of the initial microbial contamination were comparable. Strawberry samples collected from the market were also investigated. Samples were stored at 7°C, and market samples could be stored for 4 days only, while Honeoye (directly from the field) remained unspoiled up to day 6. Initial microbial contaminations of market samples were high (10^4-10^5 CFU g-1 total count, and 10^1-10^3 CFU g-1 coliforms, 10^4 CFU g-1 yeasts and moulds), and increased as a function of storage by 1-2 orders of magnitude. *Enterobacteriacea*, *Salmonellae*, *L. monocytogenes* and *B. cereus* were not detected in any of the samples. Coliforms were not found in the Honeoye.
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EMERGING PATHOGENS IN FOODS (AN OVERVIEW)

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Food-borne disease has emerged as an important and growing public health and economic problem during the last two decades. Surveys indicate that food-borne illnesses may be 300-350 times more frequent than the reported cases. They represent significant health as well as a serious economic burden. Risk assessment can be used to identify those stages in production, distribution, handling and consumption habits that contribute to an increased risk of food-borne diseases.
It is reported that known pathogens accounted for only one-fifth of the total estimated number of cases attributable to food-borne infections and about one-third of the deaths, which means that the majority of food-borne illnesses are caused by unknown, presently emerging pathogens, yet to be identified. Emerging pathogens are defined as new, re-emerging or antimicrobial-resistant infections, whose incidence in humans has increased within the past two decades or threatens to increase in the near future. Of the top 27 pathogens in the US, 13 have been identified only within the last twenty-five years. Some, like *E. coli* O157, are likely to have evolved relatively recently and may represent genuinely new pathogens. Others, such as *Vibrio vulnificus*, have only been identified recently, but may have been causing disease for years. Still others, *Listeria monocytogenes* and *Campylobacter* species have been known as pathogens for many decades, but were not known to be food-borne until recently. Increasing antimicrobial resistance is a recent trend among food-borne pathogens. The factors contributing to the emergence of pathogens, such as ecological changes (i.e agricultural practices), globalisation of the food-chain, changes in consumer demand and behaviour, changes in food technologies and the structure of the food industry, changing epidemiology of the host and the microbial changes and adaptation of microbes to the changing environment and stresses are discussed.

MOLECULAR DETECTION OF *E. COLI* O157:H7 IN CARROT JUICE FERMENTED WITH *BIFIDOBACTERIUM BIFIDUM*

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There is an increasing demand for the production of natural, minimally processed foods, which are free from preservatives and produced from natural raw materials. Fresh fruit and vegetable juices contaminated with *Escherichia coli* 0157:H7 could lead, however, to a serious illness. Heat process is able to eliminate the pathogens, but frequently it reduces the quality of the products. Therefore, new methods aiming to improve the safety of products are investigated. One of them could be the use of lactic acid bacteria with the combination of mild heat treatment. Lactic acid producing bifidobacteria decrease the pH of fruit and vegetable juices and can hereby prevent the growth of pathogenic bacteria. The aim of our investigation was to examine and compare the sensitivity of PCR-based detection techniques (real-time and conventional PCR) on artificially contaminated carrot juice.
The freshly prepared carrot juice was heat treated at 80°C for 10 minutes. After pasteurisation, we inoculated it in two different ways: (1) with the combination of *Bifidobacterium bifidum* (10^7 cell/cm^3) and *E. coli* 0157:H7 (from 10^3 cell/$cm^3$to 10^{-3} cell/cm^3) and (2) with *E. coli* 0157:H7 (10^3-10^{-3} cell/cm^3) only. In parallel, we inoculated two different types of culture media (mEC and TPY broth) with the same concentration of bacteria as control samples. To be able to compare the results of PCR detection we performed a selective enrichment in modified EC broth supplemented with novobiocine, and determined the number of colony forming units by culturing the samples on SMAC agar.
According to our results, the food matrix did not influence the efficiency of DNA isolation, but after amplification with a primer pair specific for 0157:H7, small-size extra bands appeared in the agarose gel in the case of carrot juice. After a 16 hour enrichment we determined CFU with a traditional culturing method. In samples inoculated with 10^{-3} cell/cm^3 concentration of *E. coli* 0157:H7, the cell concentration was under the detection limit. On the other hand, we could detect *E. coli* with PCR-based techniques in all samples. In culture medium in which only *E. coli* 0157:H7 was inoculated the cells could grow well, while in carrot juice we observed some decrease in the multiplication. In those cases where *E. coli* was inoculated in the carrot juice and in the culture medium together with *Bifidobacterium bifidum*, a considerable inhibition of growth took place. It can be concluded that the carrot juice and the presence of bifidobacteria have a negative effect on the growth of enterohaemorrhagic *E. coli* 0157:H7 and both types of PCR techniques were suitable to detect even the traditionally non-detectable quantity of *Escherichia coli* 0157:H7.

DETECTION OF INTRACELLULAR SIGNALLING AND CYTOSKELETAL REARRANGEMENT EVENTS IN BACTERIAL HELA CELL INVASION MEDIATED BY *YERSINIA PESTIS* PLASMINOGEN ACTIVATOR

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Yersinia pestis, the etiologic agent of plague is a highly invasive organism being able to invade non-phagocytic epithelial cells. Its plasminogen activator (Pla), encoded by the small unique virulence plasmid pPCP1, plays a pivotal role in internalisation of bacteria by HeLa cells. The aim of this study was to analyse the intracellular signalling processes and cytoskeletal rearrangement events associated with this invasion process. Wortmannin caused a 50% decrease of invasiveness at 50 nM concentration, pointing to the involvement of phopshatidyl-inositol- 4 kinase. Pretreatment with staurosporin, a potent inhibitor of protein kinases (PKs), and with genistein, a tyrosine kinase inhibitor, decreased the number of internalised bacteria about seven-fold and two-fold, respectively. Cytochalasin D, an actin

polymerisation inhibitor, the C3 exoenzyme of *Clostridium botulinum*, which is a specific inhibitor of the small GTP-ase Rho and NDGA, a 5-lipoxygenase inhibitor also involved in Rho activation, strongly reduced the number of internalised bacteria revealing the role of cytoskeletal events in the invasion process. All the tested inhibitors changed the internalisation but not the adhesion pattern of the Pla producing recombinant strain. Actin rearrangement could also be visualised with rhodamin-phalloidin staining.

STUDY OF THE EXTRACELLULAR PROTEOME OF *STREPTOMYCES GRISEUS* DURING DEVELOPMENT

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To study the cellular differentiation of industrially important streptomycetes and the role of the pleiotropic and extracellular regulatory protein factor C in the regulation of differentiation, we investigated the extracellular proteome of the following strains:
1. *Streptomyces griseus* B2682 (wild type, sporulates well, produces factor A).
2. *Streptomyces griseus* B2682-AFN (*bald* mutant, deficient in factor A production).
3. *Streptomyces griseus* B2682-AFN/pSGF4 (deficient in factor A production, transformed with the cloned factor C gene and sporulation restored).
The strains were grown either in liquid culture or on the surface of solid agar medium. The extracellular proteins were concentrated and separated on 2D SDS PAGE. The protein patterns were compared and protein spots reflecting difference among the studied strains were picked up and analyzed by MALDI-TOF-MS. The results are shown and the possible role of some of the identified proteins in cellular differentiation is discussed.

ENANTIOSELECTIVITY IN *CANDIDA ANTARCTICA* LIPASE B REACTION: TRANSITION STATES CALCULATED BY QM/MM METHODS

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Candida antartica is an easy-to-handle, well known yeast species generally applied as a biocatalyst in enantioselective esterification. Our aim was to rationalize the enantioselectivity of *Candida antarctica* lipase B (CalB) reaction by comparing the possible transition states of the two enantiomeric forms calculated by sophisticated QM/MM methods. CalB exhibits high enantioselectivity in esterifications of secondary aryl methyl carbinols. The degree of enantioselectivity of CalB-catalyzed acetylations of 1-phenylethanol, 1-cyclohexylethanol and 1-(benzofuran-2-yl)ethanol was determined experimentally by chiral GC. Until now the most realistic calculations were performed by calculating the energy difference between tetrahedral intermediates for the two enantiomers [1]. For each enantiomer, two transition states (one for the alcohol-acylenzyme complex and one for the enzyme–acylester complex) are needed to be calculated, the real energy difference can be evaluated by comparing the highest energy for the *S* enantiomer with the highest energy of the *R* enantiomer. For the calculation of transition state energies, the ONIOM (QM/MM) methods were used by optimizing a

10Å area around the substrate, while the substrate and the most important residues were calculated using ab initio and DFT methods.

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MICROBIAL COMMUNITIES OF THE SEDIMENT OF TWO HUNGARIAN SHALLOW LAKES (HÉVÍZ AND BALATON) STUDIED BY CHEMOTAXONOMICAL METHODS

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Lake Hévíz is one of the most important curative waters of Hungary with a high organic content. The importance of Lake Balaton is also undoubted as it is the largest natural lake of Europe with a touristical importance. 5 points of the sediment of Lake Balaton (Siófok, Balatonakali, Szigliget, Zala-issue and Keszthely-bay) and 2 points of the sediment of the Lake Hévíz (at entrance and near the crater) were sampled with Hargrave-equipment. Samples (20 g of each) were taken into Bligh and Dyer solution, cooled and stored at –20°C for the time of processing. Lipid constituents were extracted in two steps then the determination of total biomass was carried out. For the separation of lipid components, the extracted lipids were separated on BB C18 column (Merck) using chloroform, acetone and methanol solvents, respectively. The chloroform fraction (containing respiratory quinones and also contaminants) was concentrated with a rotary evaporator at 37°C and then quinones were purified with thin layer chromatography on Kieselgel 60 F254 sheets. The quinones were identified with HPLC (Pump 2248, Uvicord SII detector) using the quinone profiles of authentic bacterial strains as standards. The methanol fraction (containing fatty acids) was similarly concentrated with a rotary evaporator at 37°C and then fatty acid methyl esters were produced. The analysis of fatty acids was carried out by HP 5890 gas chromatograph with HP1 capillary column using BAME (Sigma) standard. For the comparison of our chemotaxonomical data, principal component analysis (PCA) was carried out using the Syntax 2000 software package. The highest biomass values were measured at the Hévíz entrance (50.78m gC/g dry sediment) and at the Balaton Zala-issue (47.26 m gC/g dry sediment) samples. By their chemotaxonomical markers the two lakes grouped separately, but the different sampling sites from the same lakes grouped together. Except for the Balatonakali sediment sample, the dominance of prokaryotic microorganisms was characteristic in all cases. In the Hévíz Lake samples the markers of sulphate reducing bacteria, different Actinobacteria, Proteobacteria and microfungi occurred. *Bacillus* specific markers could also be detected. The ratio of Gram negative bacteria in the entrance sample was twice of the crater one. Differences in the sampling sites occurred in the Actinobacteria content (markers of different Actinobacteria could be detected at different sampling sites) and among microfungi. Several quinone compounds remained unidentified from these samples. In the 5 samples of the sediment of Lake Balaton the main chemotaxonomical markers agreed, differences could only be detected in minor components. Thus, in all samples the markers of the genus *Bacillus*, different Gram negative bacteria and other, universal markers could be detected. Differences among samples occurred in the microeukaryotic content (Diatomes and microfungi), different Actinobacteria and the presence of markers of Enterobacteriaceae was found in 2 samples.

DELTA1-DEHYDROGENATION OF STEROIDS WITH IMMOBILIZED AND CELL-FREE ENZYME-SYSTEMS

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The microbiological Δ1-dehydrogenation of steroids has been used in the pharmaceutical industry for many decades. A number of publications on the 3-ketosteroid-1-dehydrogenase enzyme (EC 1.3.99.4) isolated from different microorganisms can be found investigating its active center, mode of action and kinetic properties [1]. These enzymes catalyse the formation of a double bond in ring A of steroids at position 1 [2]. The inducible enzyme molecules adhere strongly to cell particles, thus they are difficult to be solubilized. The coenzyme is characteristically FAD and can be removed only irreversibly; activity can be restored only by providing artificial electron acceptors. Many teams aiming to purify and immobilize this enzyme found this task difficult because of the instability problems mentioned before.

We have investigated the 3-ketosteroid-1-dehydrogenase obtained from *Arthrobacter simplex*. We tried to stabilize the enzyme activity by immobilizing the induced cells with various carriers, considering the possibility of their repeated use in dehydrogenating reactions. The preferred matrixes of immobilization were calcium-alginate and polyacrylamide; the bioconversion efficiency of these enzyme systems were compared to that of free intact cells and cells pretreated with Triton lysis buffer. Immobilizations were made following the methods known from relevant publications. Bioconversions were performed at room temperature in 100 ml shake flasks agitated effectively. Cortisol in organic solvent as the substratum of the enzyme was added to the system; the bioconversion efficiency was investigated by monitoring the production rate of prednisolone spectrophotometrically. The proportion of the dehydrogenated product was determined by the double wavelength method based on the spectral difference of cortisol and prednisolone.

The rate of bioconversions performed by our immobilized preparations differed a little; the enzyme activity retained in the polyacrylamide gel slightly exceeded the activity measured in the alginate bead system, presumably because of better conditions for diffusion. Comparing bioconversion rates of systems containing the same enzyme activity in untreated intact cells or ones pretreated with Triton lysis buffer with that of the immobilized systems, an 80% decrease could be observed in the latter. The complete conversion of added substrate in the immobilized system required approximately 2 hours.

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RELATIONSHIP BETWEEN SURVIVIN PROMOTER POLYMORPHISM AND HUMAN PAPILLOMAVIRUS ASSOCIATED CERVICAL CARCINOMA

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Cervical carcinoma is one of the most frequent cancer types among women worldwide. Human papillomaviruses (HPV) are thought to be involved in the development of the disease.

Survivin, a unique antiapoptotic factor, plays an important role in both cell cycle regulation and inhibition of apoptosis. It is undetectable in most terminally differentiated normal tissues but is expressed in embryonic and foetal organs. There is significant correlation between survivin and the presence of many human cancers, such as cervical carcinoma.

Several nucleotide polymorphisms were identified within the promoter region of the survivin gene, one of which is located at the CDE/CHR repressor binding site. This polymorphism seemed to be as-

sociated with the overexpression of survivin in a number of cancer cell lines. In this study, we focused on the relationship between the presence of the survivin promoter polymorphism and the HPV associated cervical carcinoma.
Using Restriction Fragment Length Polymorphism (RFLP), we could determine the nucleotide variants at the polymorphic site within the survivin promoter region in Hungarian women with cervical carcinoma and in a control population. In addition to RFLP, PCR-based Single-Strand Conformation Polymorphism was used to detect the same sequence variation. Data from the two methods correlated completely. We are planning to investigate the allele frequency (at the CDE/CHR repressor binding site) in HPV-positive premalignant lesions compared to HPV-negative samples, to shed light on the importance of the survivin promoter polymorphism in connection with cervical carcinoma.

BACTERIAL SPECIES DIVERSITY IN A KISKUNSÁG SODA LAKE (HUNGARY) EVALUATED BY A POLYPHASIC APPROACH

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Soda lakes represent the most alkaline naturally occurring environments on the Earth, with extremely high pH values and sometimes saline conditions. These sites are mostly characterized by large amounts of $NaCO_3$. The soda lakes of Kiskunság National Park are extremely shallow, alkaline and moderately saline water bodies that may dry out completely by the end of the summer. This study aimed to reveal and compare the hitherto unknown bacterial species diversity of the lake Kelemenszék of KNP by applying traditional cultivation-based techniques and cultivation-independent molecular biological and chemotaxonomic analysis. Sediment samples were taken from the upper 3-5 cm layers in 2002, 2003 and 2004. Serially diluted samples were plated onto different media and bacterial strains were investigated for their morphological, biochemical and ecological tolerance characters and grouped by hierarchical cluster analysis. Phenon representatives were identified by 16S rDNA sequence analysis. The species diversity of the May 2003 sediment sample was also studied by cloning of the 16S rRNA gene. Through the chemotaxonomic investigations determination of total biomass, isolation and characterization of respiratory quinones and fatty acids were carried out. 119 bacterial strains were isolated and on the basis of 35 phenotypical data a hierarchical cluster analysis was carried out. More than 90% of the strains were Gram positives and the majority (85%) had aerobic respiratory metabolism. Except for the hydrolysis of biopolymers, the strains rather showed inactivity in the biochemical-physiological tests. Most of the strains proved to be facultative or obligate alkaliphilic and moderately halophilic. Except for two strains which were closely affiliated with the species *Halomonas desiderata* (gamma Proteobacteria), the low G+C Gram positive Firmicutes (members of genus *Bacillus* and related species: *B. cereus, B. pumilus, B cohnii, B. pseudofirmus, B. alcaliinulinus* and *Marinibacillus marinus*) were predominant. The phylogenetic analysis of the partial 16S rRNA gene sequences indicated that the majority of the clones (67%) were related to four subdivisions of Proteobacteria. Other clones showed generally low (88-96%) sequence similarities with Nitrospira, Planctomycetes, Gemmatimonadetes, Actinobacteria, Bacteroidetes and Chloroflexi. Among the identified clone sequences besides the metabolic potential of the cultivated aerobic chemoorganotrophic bacteria (*Bacillus* species), microorganisms with a wide-range or specific metabolism were also found. Biomass value of the sediment of the lake was not too high. The ratio of higher taxa based on chemotaxonomic analysis was 45% Gram negatives, 34.3% Eukaryotes, 16.7% Gram positives and 4% unidentified. Among eukaryotic microorganisms microscopic fungi and protists were present. Several *Bacillus* specific markers were detected; these

bacteria gave 72.4% of all Gram positives. Actinobacteria specific markers could also be found. From Gram negatives the presence of family Enterobacteriaceae and Leptospiraceae were obvious. Characteristic markers of Cyanobacteria were also detected.

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APPLICABILITY OF MATHEMATICAL MODELS IN DEFINING THE KINETIC BEHAVIOUR DISTINCTION AMONG MICROBIAL STRAINS

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Generally, the behaviour of any microorganism is determined by its genetic properties, and depends on its culture conditions. Since microbial culture kinetics can be well described by appropriate mathematical models, one can consider that mathematical models can be applied to define the kinetic behaviour distinction among microbial strains. We supposed that the experiments referring to oxytetracycline biosynthesis with different *Streptomyces rimosus* derivative strains could be an excellent example to demonstrate the validity of the above statement. In our first series of experiments, we compared the biosynthetic activities and the growth kinetics of microbial colonies of several *S.rimosus* mutant strains cultivated on agar plates. Later, we focused our interest on the two chosen strains, in order to express their differences mathematically with respect to their colony growth and antibiotic biosynthesis kinetics. Finally, we decided to study the behaviour of the three selected *S.rimosus* derivative strains under different culture conditions, with an aim to define strain distinction parameters. Mathematical models based on the three-dimensional growth concept, (*dgx/dt = k1.gx2/3.(gx/(kx+gx)) - k2.gx*) describing the growth, substrate uptake and antibiotic biosynthesis kinetics of microorganisms, were developed. Computer simulation was applied to verify the applicability of the mathematical models. The excellent agreement of computer simulation with experimental data confirmed the hypothesis that the kinetic parameters can be successfully applied to define the behaviour distinction among different *S.rimosus* strains. In the case of the selected three strains, *S.rimosus* R6-500, *S.rimosus* MV9R-1 and MV9R-2, it was established that they can be distinguished by their growth kinetic parameters *k1* and *kx,* with the same *k2* value, by their substrate uptake kinetic parameters (maximal specific carbohydrate uptake rate, *qsub,* substrate uptake inhibition constants and the rate of active biomass concentration reduction) and by their antibiotic biosynthesis kinetic parameters (maximal specific antibiotic biosynthesis rate, *qp,* antibiotic formation inhibition constant, inorganic phosphorus effect and the rate of active biomass concentration reduction). The strain *S.rimosus* R6-500 showed to be superior with respect to all kinetic parameters; the strain *S.rimosus* MV9R-2 showed to be slightly inferior to it, whereas the strain *S.rimosus* MV9R-1 showed to be inferior with respect to the both mentioned strains, especially because it showed pronounced active biomass reduction rate under all investigated culture conditions. Based on these and our corresponding previous results, one can conclude that appropriate mathematical models can be recommended to be used in defining parameters of microbial behaviour distinction among different microbial strains.

ARBUSCULAR MYCORRHIZA AS PART OF THE RHIZOSPHERE AT HEAVY METAL AND SALT STRESS

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Soils containing heavy metals can carry specific vegetations. Some of the plants of such places, the metallophytes, are colonized by arbuscular mycorrhizal fungi (=AMF). This was investigated in some detail for zinc violets, *Viola lutea* ssp. *calaminaria* and *V. lutea* ssp. *Westfalica*, which are endemic in Central Europe. An isolate of the arbuscular mycorrhizal fungus *Glomus intraradices* was obtained from the roots of the zinc violet of the stand Breinigerberg near D-Aachen, and this isolate consistently confers heavy metal tolerance to plants [1]. Element localisation studies showed that AMF-colonized roots contain less heavy metals than controls after growth in a heavy metal soil. The heavy metals that inevitably reach the inside of the roots are concentrated in the inner parenchyma cells, where the fungal structures reside [2]. Gene expression studies indicated that the transcript level of some specific metallothionein and broad range heavy metal transporters (N*ramp* family) are down-regulated upon colonization of tomato with AMF. On the fungal side, genes with products involved in stress alleviation are up-regulated [3]. Mycorrhizal fungi offer good perspectives for phytoremediation of heavy metal contaminated soils.

More than 7% of the soils worldwide is polluted by NaCl. Plants of diverse salt marshes are also colonized by AMF. Indeed, the salt aster, *Aster tripolium*, is one of the strongest AMF colonized plants [4]. Soils of salt marshes, also in Hungary, contain a very high percentage of spores of one single AMF, *Glomus geosporum* [5]. In contrast, roots of *Aster tripolium* and of other halophytes are not so much colonized by *G. geosporum* or *G. intraradices*, but by a group of AMF that is unculturable and have only been described by sequences (to be published) so far. Gene expression studies indicated that aquaporins and so many Na+/H+ transporters are involved in NaCl stress alleviation [6]. An AMF isolate that confers salt stress to plants has not yet been obtained by us.

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GENERATING AND STUDYING THE INFECTIVITY OF COAT PROTEIN CHIMERAS OF POTATO VIRUS Y

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Potato virus Y (PVY) is one of the most important plant viruses infecting several food crops of the *Solanaceae*. To learn more about the role of CP in the infection process, the coat protein (CP) coding region of the N strain of PVY was exchanged with the corresponding region from other strains of PVY. This study is based on the full-length infectious cDNA clone of PVY-N605 created by Jakab *et al.* (1997). To enhance the stability of the clone in *E. coli*, three introns were introduced as described for *Pea seedborne mosaic virus* by Johansen (1996). The introns are excised via splicing mechanisms *in planta* resulting in infectious viral RNA. The different CP coding regions were derived from PVY-NTN and PVY-O. *E. coli* strain NM522, and standard cloning techniques were used to generate chimeric clones. After testing the infectibility by particle bombardment of the chimeras, a host plant screen was carried out.

Two chimeras were generated carrying the CP coding region and the 3'NTR of the PVY-NTN and O strains in a PVY-N background. The chimeras revealed no mutations by sequencing of the exchanged region, and remained infectious. Symptomatology of the virus „strains" (three parental viruses and two infectious chimeras) was compared on fourteen host plants; in most cases there were no significant differences except for *Physalis floridana,* where symptom differences occurred between the parental viruses and their chimeric derivatives. The PVY-N/NTN chimera showed symptom similarity to PVY-N, while the PVY-N/O chimera produced similar symptoms to that of the „CP parent" PVY-O. The experiments demonstrated that the intron containing PVY full-length clone is suitable for further manipulations. Exchange of the CP-3'NTR region between different strains of the same virus was accomplished without a loss of infectivity. Based on the symptoms on *Physalis floridana* the CP and the 3'NTR are probably not the only determinants for symptom development of PVY-NTN, however, in case of PVY-O it could play a more important role in this process.

ADHESIVENESS AND MIGRATION OF *ESCHERICHIA COLI* AND *ENTEROCOCCUS FAECALIS* CLINICAL ISOLATES OVER FOLEY CATHETERS

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At present, a single catheterisation of urinary bladder is known to be associated with around 6% risk of infecting the urinary tract, while every day of residence of a Foley catheter in urinary bladder is accompanied by around 5% risk of the infection. Considering the above, a microbiological evaluation of the applied urological catheters seems very important. In present study migration of the most frequent uropathogenes (*E. coli* and *E. faecalis*) over Foley catheters and their adhesive properties were examined. The studied material included 20 isolates from patient urine, including *E. coli* clinical strains identified using ID 32 GN (bioMerieux) test and 10 clinical strains of *E. faecalis* identified using Rapid ID 32 STREP (bioMerieux) test. The studies were performed using various urinary catheters (hydrogel-coated latex, HL; polyvinyl chloride, PVC; silicone-coated latex, SL; and all-silicone, S). The migration of bacteria over catheter sections was examined using a laboratory model in which 24h migration of *E. coli* and *E. faecalis* across sections of selected catheters was tested on TSA containing plates. The obtained results were expressed in mean migration indices. Crystal violet staining method was used For the evaluation of 24h adhesion to the surface of the applied catheters, while attachment of studied isolates was measured by absorbance (A) readout at λ=540 nm.
The obtained results demonstrated that migration index and absorbance (A) of *E. coli* strains were dependent upon the applied type of catheters and ranged from 0 and A=0.260 (for S catheter) to 85 and A=2.110 (for HL catheter). In cases of *E. faecalis* strains the migration index for any catheter was 0, while A ranged from 0.150 for the S catheter to 1.760 for the HL catheter. The obtained results permitted to conclude that bacterial migration through urinary catheters and adhesion to their surface are influenced by the material from which they are formed. Silicone (S) catheters seem least susceptible to colonisation with the most frequent uropathogenes.

COMPARISON OF SMPB PROTEINS FROM INTRACELLULAR PATHOGENS

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SmpBs are small RNA-binding proteins, components of the conserved tmRNA-SmpB system, which has the dual role of releasing stalled ribosomes from damaged messenger RNA, and adding a tag for destruction to stalled protein. In bacteria, this unique translational process, called trans-translation, is a universal quality control mechanism.
6His and FLAG-tagged SmpB (the product of CpB0345) were cloned from *Chlamydophila pneumoniae* TW-183, expressed in *Escherichia coli* and they were partially purified. It is one of the largest SmpBs among the intracellular pathogens (19 kDa, 165 amino acids) and exhibits 48% identity with *Mycobacterium tuberculosis* H37Rv SmpB. The CLUSTAL W program was used to draw an evolution tree on the basis of the SmpB sequences. Only slight agreement was observed with the phylogenetic classification based on the small-subunit ribosomal RNA. SmpB proteins and trans-translation can be regarded as promising targets for the development of new inhibitors in the fight against bacterial infections.
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CAROTENE PRODUCTION WITH GENETICALLY MODIFIED *MUCOR CIRCINELLOIDES* STRAINS

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Carotenoids are one of the most important groups of natural pigments. They are used in the food, pharmaceutical and cosmetic industries and also as feed color additives. The progress in the cloning and analysis of microbial carotenogenic genes opens up the possibility of modifying and engineering the carotenoid pathway in different microorganisms. *Mucor circinelloides* is a *b*-carotene producing zygomycete, frequently involved into the study of the carotenogenesis in fungi. *b*-Carotene is an isoprene-type chemical compound and its production can be increased by over-expression of the genes encoding the enzymes of the isoprene biosynthetic pathway.
In the present study, expression vectors containing the known isoprenoid genes of *M. circinelloides* (e.g. *isoA* encoding farnesyl pyrophosphate synthase and *carG* encoding geranylgeranyl pyrophosphate synthase) were introduced into a double auxotrophic (*leu*-, *ura*-) strain of *M. circinelloides* by polyethylene glycol mediated transformations. Expression vectors contained the *Mucor leuA* or *pyrG* genes as selection markers. All studied transformants proved to be autoreplicative: aintenance of the selective conditions was necessary that the transformants retain the plasmids. Carotene content in the resulted transformants and in the wild-type strain was measured by high-performance liquid chromatography. In comparison with the original strain, transformants containing plasmids with *isoA* or *carG* produced about 1.4 or 1.7 fold more carotene, respectively. In the co-transformant strains carotene production increased about 2 fold.
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MULTIPLE MYELOMA (MM) PATIENTS AND PERINATAL TRANSMISSION OF HUMAN HERPESVIRUS-8 (HHV-8) INFECTION

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It has been proposed that HHV-8 (Kaposi's sarcoma herpesvirus) might possess a promoting effect in the development and progression of monoclonal gammopathies. The diversity of the molecular and serological diagnostic procedures, however, resulted in a great deal of contradictory findings and publications. Primary infections may be diagnosed using simple PCR methodology, since the plasma samples are usually positive if active virus replication is present in the patient. Only nested PCR is sensitive enough for the detection of virus carrier state. One of the main contradictions of the field is that series of patients shown to be carriers of HHV-6 and HHV-8 using validated nested PCR tests had been found to be seronegative with commercial or "in house" serological methodology. Several attempts have been made in our study to address this controversy. Patients suffering from Non-Hodgkin lymphomas (NHL) were also tested for the presence of the DNA of other herpesviruses (EBV, HHV-6 and human cytomegalovirus = CMV).
The presence of HHV-8 nucleic acid could be detected in 25 of 62 MM patients (40.3 %). The rate of positivity among 28 NHL patients was only 10.7 %. In contrast to these, the rate of positivity (among the 69 MM-patients) to CMV, EBV and HHV-6 DNA was found to be 5.1 %, 46.7 % and 21.7 %, respectively. The corresponding results for the 44 NHL patients were 3.4 %, 55.6 %, and 13.6 % to EBV, CMV and HHV-6 DNA, respectively. Our data indicate a possible involvement of HHV-8 infection in some - thus far undetermined - aspect of MM pathogenesis.
In order to see a healthy control population, serum samples of 70 pregnant women on the day of the delivery have been tested for the presence of HHV-8. Seven of them (10 %) were found to be positive, indicating that the NHL group of patients has not been suffering from a more serious immunosuppression than that of the pregnant women.
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HUMAN HERPESVIRUS 6A (HHV-6A) SUPPRESSES HUMAN IMMUNODEFICIENCY VIRUS (HIV) REPLICATION IN HUMAN MACROPHAGES

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HHV-6A frequently reactivates in HIV-infected patients, it has been proposed as a cofactor in AIDS progression. In order to study the interaction between HHV-6A and HIV-1, we focused on macrophages, which have importance in HIV-1 and also in HHV-6 infection. For this purpose, monocytes were isolated from peripheral blood, and monocyte-derived macrophage cultures were established. Differentiated macrophages were infected with HHV-6A or HIV-1, or simultaneously with both viruses. We examined the virus production of the differently infected cultures.

Replication of HIV-1 is significantly suppressed in coinfected cultures, contrary to macrophages infected with HIV-1 alone. According to previous studies, HHV-6A infection was not productive in macrophages as revealed by infective titration. We observed the lack of p150 capsid polypeptide of HHV-6 by immunofluorescence staining. As a result of HHV-6A infection, coinfected macrophages secreted considerable amount of interleukin-8 (IL-8) and RANTES. These chemokines may influence the susceptibility of macrophages to HIV-1 infection by altering the sensitivity of HIV-1 coreceptor, CCR5 and also the expression level of the receptor. We determined that RANTES-elicited intracellular Ca^{2+} mobilization is significantly lower in HHV-6A infected macrophages than in uninfected cells. Consequently, HHV-6A infection may reduce the sensitivity of CCR5 receptors to R5 variant of HIV-1. Nevertheless, the expression level of CCR5 is markedly reduced on HHV-6 infected macrophages compared with uninfected cells using flow cytometry analysis.Addition of exogenous IL-8 and RANTES also suppressed HIV-1 production in macrophages infected with HIV-1 alone, which suggests that these chemokines may play an important role in the interaction between HHV-6A and HIV-1. Summarizing our results, we determined that HHV-6A suppresses HIV-1 replication in macrophages, the susceptibility of these cells to R5 variant HIV-1. We hypothesized that HHV-6A-induced IL-8 and RANTES may have importance in these processes.

TAXONOMIC IDENTIFICATION OF YEASTS COLONISING GRAPES DURING NOBLE ROTTING IN TOKAJ

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Tokaj is one of the major European wine regions, where botrytized wines can be produced. In these regions, particular climatic conditions permit *Botrytis cinerea*, a pathogenic fungus, to launch a specific process. The grapes infected by *B. cinerea* develop what is called noble rot or aszu-formation. Several microbiological examinations suggested that noble rotted berries could be colonised by additional microbes including yeasts. To investigate the yeast microbiota, we collected healthy and botrytized berries in three different vineyards at the vintages in years 2002-2004, and isolated yeasts from the samples. The taxonomic identification of the isolates was done by conventional yeast identification methods based on morphology, sporulation, utilisation of carbon sources, and nitrogen sources, tolerance to ethanol, 1 % acetic acid, growth at various temperatures, etc.

The molecular analysis was done with representative strains of the species found. The PCR-RFLP of the ITS1-5.8S-ITS2 region and the sequencing of the 26S region of the rDNA confirmed that most isolates belonged to *Kluyveromyces thermotolerans*, *Candida stellata*, *Candida zemplinina*, *Candida sorbosivorans*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Metschnikowia viticola*, *Cryptococcus laurentii*, *Cryptococcus albidosimilis*, *Cryptococcus magnus var. magnus*, *Cryptococcus adeliensis*, *Rhodotorula nothofangi*, *Rhodotorula glutinis*, *Rhodosporidium kratochvilovae* and *Aureobasidium pullulans*.

THIRD GENOME SIZE CATEGORY OF AVIAN PARAMYXOVIRUS SEROTYPE 1 (NEWCASTLE DISEASE VIRUS) AND EVOLUTIONARY IMPLICATIONS

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The goal of the study was to find relationship between molecular patterns and virus evolution. Therefore, the complete genome sequence of two distinct apathogenic Newcastle disease virus (NDV) strains was determined and a third genome size category, containing 15 198 nucleotides, was recognized. Phylogenetic analysis revealed that two major separations, resulting in three genome size categories that occurred during the history of NDV. An ancient division in the primordial reservoir (wild waterbird species) led to two basal sister clades, class I and II, with genome sizes of 15 198 (due to a 12 nucleotide insert in the phosphoprotein gene) and 15 186 nucleotides, respectively. Ancestors of only class II viruses colonized chicken populations and subsequently converted to virulent forms. These took place more than once and resulted in an early lineage [including genotypes I-IV and H33(W)] with genome size of 15 186 nucleotides. A second division occurred in the 20th century in the secondary (chicken) host.
This gave rise to the branching-off of a clade (including recent genotypes V-VIII consisting of only pathogenic viruses) with the concomitant insertion of six nucleotides into the 5' non-coding region of the nucleoprotein gene, thereby increasing the genome size to 15 192 nucleotides.

THE ROLE OF ALKALIPHILIC BACTERIUM SPECIES IN THE COMPOST PRODUCTION ENRICHED WITH WOOD ASHES

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Bacillus species are fulfilling the needs for physical parameters of compost-making. In theory, such bacterium species can occur in the base materials of compost, however, their enrichment in a compost pile containing wood ashes would prolong the duration of compost production, resulting in an economic loss. For *Bacillus* starter expriments, bacilli have been isolated from the sediments of Lake Fertő, Lake Velencei, Bödi-szék and Kelemen-szék, and identified as *B. halmapalus, B. firmus, B. pseudofirmus, B. cohnii, B. alcalophilus.* Based on preliminary experiments two strains of them were used. The ratio of wood ashes has been determined so that the pH values of base material mixtures (and that of ripened compost) will not surpass pH 8,5, which is a pre-requisite of e.g. agricultural use. On the basis of the results of the experiments it can be concluded that the compost can be blended with 2% of wood ashes, whereas the sewage-sludge base material can be mixed with 12% of wood ashes. It has been shown with solid stage fermentation experiments that the two chosen *Bacillus* strains added to organic waste base materials outcompeted their natural bacterium biota in the presence of wood ashes.
The above pre-experiments suggest that the examined alkalohalophilic strains (being able to grow even at extreme pH conditions, though their growth optimum is at pH 9) regulate the pH of their environment through their metabolic products.

THE METAL CONTENT AND THE MICROORGANISMS ON THE PHYLLOSPHERE OF RAGWEED PLANTS (*AMBROSIA ELATIOR* L.) IN RUDERAL ENVIRONMENTS

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The studies focused on "total" cadmium, copper, nickel and zinc content of the soil in three ecologically distinct areas - species composition, metal contents and formation - as well as the corresponding metal contents in ragweed plants in the same areas, and the microorganisms in the leaves in the years 2000–2003. Changes in the individual number of microorganisms on the leaves and in the air were compared. The results were related to the base year of 2000, as well as to the figures obtained for Investigation Site I (control site). Metal content related to average dry weight, the soil of Investigation Site I and Site II. (communal landfill site) is not contaminated with metals, whereas the soil of Site III. (industrial galvanic-sludge disposal site) can invariably be characterized as contaminated with metals. The results of the investigations implied that, parallel with the increase in the examined metal content of the soil, significant differences occurred between the metal contents of the soil and those of the plant organs (SD=5%). At the investigation sites not contaminated with cadmium, copper, nickel and zinc, ragweed tends to accumulate metals in the roots, but as the extent of contamination becomes more serious, the average content of cadmium (0,33 – 1,49 mg/kg), copper (7,27 – 11,70 mg/kg) and zinc (53,20 – 304,00 mg/kg) are higher in the leaves than in the roots. The leaf surfaces of ragweed plants are highly segmented, and have plenty of leaf hairs, veins and stomata. Microorganisms tend to accumulate along leaf veins and on the surfaces of leaf hairs in the largest number., The germ count of bacteria showed positive correlation both on the upper and lower surfaces of leaves, in all the three studied years with the increase in the metal content of the leaves There were certain differences observed in the germ count of bacteria, thread fungi and yeasts on the upper and lower surfaces of the leaves. The germ counts were found to be larger on the upper surfaces of leaves than on the lower part. From ragweed leaf surfaces, primarily *Enterobacter agglomerans Erwinia sp*., *Pseudomonas sp*., *Chryseomonas luteola*, *Bacillus sp*., *Bacillus cereus* bacteria, *Cryptococcus sp*. yeast, *Alternaria sp*. and *Penicillium sp*. thread fungi could be isolated. The obtained results also revealed that when metal contents in the leaves showed an upward trend, the species diversity of bacteria and thread fungi decreased, while the individual numbers of microorganisms increased. Variations in the individual numbers of bacteria in the air and on ragweed leaf surfaces reflected positive correlation in all examination years. The significant differences suggested that the individual number of microorganisms in the ragweed phyllospheres were influenced by the increasing metal contents of plant leaves.

PENTOSE CATABOLISM IN SAPROPHYTIC AND PATHOGENIC FUNGI

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Catabolism of pentoses, specifically L-arabinose and D-xylose, is an important pathway for many fungi due to the abundance of these carbon sources in nature. This pathway has so far been mainly analysed in *Aspergillus niger* and *Trichoderma reesei*. Recent data of the regulation of this pathway in *A. niger* have been reported including a model for the relative contribution of the pathway specific regulator (AraR) and the xylanolytic regulator (XlnR).
Initial studies into the role of the pentose catabolic pathway in the rice pathogen *Magnaporthe grisea* demonstrated a role for this pathway during infection, and identified an L-arabinose reductase that is

specific for this fungus. Finally, an analysis of the presence of this pathway in fungi of which a genome sequence is available is presented.

DETECTION OF HUMAN PAPILLOMAVIRUSES BY HCA, PCR AND RT-PCR METHODS

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Our aim was to compare the nucleic acid hybridization assay with PCR and real-time PCR methods for the detection of human papillomaviruses (HPV), which are regarded as the most important agents of cervical carcinoma.

Cervical samples were collected from previously screened, known HPV-positive and HPV-negative fertile women. Sampling, sample transport and HPV DNA determination were performed according to the kit instructions of Digene HPV HCA, and the number of virus copies were calculated. HPV PCR was performed by a traditional method, and real-time PCR (SybrGreen 1) in a LightCycler. PCR and real-time PCR results were controlled by agarose gel-electrophoresis.

40 of the 69 cervical samples were positive with HCA, 33 with PCR and 43 with the real-time PCR method. 27 (67.5%) of the 40 HCA high-risk positive samples contained > 20.000 virus copies in each 50µl cervix sample. There were between 5,000 and 20,000 copies in five (12.5%) samples. In eight (20.0%) samples, the number of HPV virus copies was <5,000.

HCA is a conventional, reliable and extensively used method in many laboratories, but it is work and time-consuming (6-8 hours). Traditional PCR was less sensitive than HCA. The sensitivity of the real-time PCR proved to be the highest. The SybrGreen method applied in the real-time PCR involves DNA staining, which is not suitable for quantification. The TaqMan method for quantification is planned to be introduced in our laboratory.

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DETERMINATION OF HSV SEROPREVALENCE IN DIFFERENT SOUTH-HUNGARIAN POPULATION GROUPS

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The objective of this study was to determine type-specific HSV-2 and HSV-1 seroprevalence in representative samples of women and men in the Szeged region in South-Eastern Hungary.

A total of 1500 convenience-based samples were included. Overall, the study group included 249 students at the University of Szeged, 153 abortion patients, 287 antenatal clinic attendees, 46 *in-vitro* fertilization clients, 238 blood donors and 527 health care professionals. Participants were interviewed for information about demographic and life-style factors. Blood samples were collected to determine type-specific serum IgG antibodies against HSV-2 and -1, using Focus Diagnostics ELISAs. HSV-2 and -1, seropositivity and 95% confidence intervals (CI) were calculated.

The HSV-2 seroprevalence in the surveyed study groups ranged from 6% (95% C.I.: 3.4-9.7%) to 22.6% (19-26%), with a pattern consistent with a higher HSV-2 seroprevalence at older ages. The levels of HSV-1 serum antibodies increased sharply at younger ages, and were detected in the major-

ity of the sampled adult population groups, ranging from 49.9% (95% CI: 43-56%) to 82.8% (77-87%) overall in the populations surveyed.

Dates	Population group	Mean age (range) in years	HSV-1 (%)	HSV-2 (%)
May – June 2004	College students	21.9 (18–53)	49.9	6.0
Feb. – Dec. 2004	Abortion patients	29.5 (14–46)	76.5	16.3
Jan. - Dec. 2004	Antenatal attendees	29.5 (16–44)	79.1	13.6
Mar. - Oct. 2004	*In-vitro* fertilization clients	32.3 (22–47)	80.4	17.4
Jan. - Dec. 2004	Blood donors	34.6 (18–69)	82.8	15.1
Sept. – Nov. 2004	Health care personnel	41.2 (18–79)	77.0	22.6

The level of HSV-2 seroprevalence varied appreciably between the representative samples of these different population groups in Hungary (6-23%), yet appeared to be dependent upon the mean age of the study population. A high HSV-1 seroprevalence of over 75% was consistently found in the surveyed adult groups. Data are currently needed on the time trends of HSV-2 and 1-seropositivity in Hungary.

DETECTION OF CANINE DISTEMPER VIRUS STRAINS BY POLYMERASE CHAIN REACTION TECHNIQUE AND THEIR PHYLOGENETIC ANALYSIS

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Despite the intense vaccination procedures applied in developed countries, canine distemper is still one of the most serious threats of the susceptible carnivore populations. This is why a reliable and fast diagnostic technique, such as polymerase chain reaction (PCR), is extremely important, influencing both the outcome and the prognosis of this disease. It is also valuable in forming a proper diagnosis from poorly preserved field samples taken from animals died of distemper. This molecular biological technique has been used to develop new differential diagnostic tests, while sequence data of the amplified regions are being used to study virus epidemiology at the molecular level and to determine the phylogenetic relationship among the different strains of the canine distemper virus (CDV).
More than 100 samples collected from dogs showing respiratory, gastrointestinal and neurological signs suggesting CDV infection were examined. The samples consisted of urine or blood collected from clinically ill patients and sent to our laboratory by clinicians from various veterinary clinics throughout Budapest or they were collected during necropsy from determined organs (lungs, liver, urinary bladder, urine, lymph nodes, brain etc). For diagnostic purposes, a primer pair specific for a 400 bp long segment of the conservative region of the large polymerase region (L) of the CDV genome was designed. Due to the conservative aspect of the amplified segment, this primer pair can be used for the detection of all strains of CDV currently present in Hungary. For the phylogenetic analysis, a 1100 bp long variable region of the haemagglutinin (H) gene of the CDV genome was targeted. The amplicons from the later region were sequenced in both directions using the appropriate primers. The resulting sequences were compared to the sequences available in relevant genetic databases. Our results indicate that the strains present in Hungary are closely related to strains that were reported in other countries of Europe, such as Italy and Germany. The main differences were located in the H

gene segment, indicating that this region of the viral genome is highly variable and can be used in comparative phylogenetic analysis of the related strains and viruses.

ASCERTAINMENT AND ANALYSIS OF THE RISK FACTORS OF HSV USING FEATURE-SELECTION ALGORITHMS

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The number of infections and diseases involving herpes virus is increasing year by year in Hungary. Epidemiological studies have not yet been published on this topic. Our objectives were to reveal the scale of infection in different groups, and to determine the causative agents.
Between 1 January and 31 December 2004, we processed 1500 blood samples in the Virus Laboratory of the Department of Clinical Microbiology. The sources of the samples were six different groups: students at the University of Szeged, health care workers (HCWs), participants in the in vitro fertilization programme at the Kaali Institute (University of Szeged), participants in positive and negative family planning sessions at the Department of Obstetrics and Gynaecology, and donors attending the Regional Blood Centre of the National Blood Bank in Szeged.
We created a database containing data on the various groups and serological results in order to determine the most important risk factors relating to HSV infections for the different groups in the database. From this database, we selected possible causative features. The pre-processed data were examined for possible risk factors using various data mining methods. We currently have results from the overall database and from data on the patients at the Department of Obstetrics and Gynaecology. The data mining methods yielded a set of parameters with which the learning algorithm used was the most accurate. Concerning the overall database, in the case of HSV-1 the learning algorithm provided a precision of 66.2% and 5 risk factors; in case of HSV-2 the precision was 88.3% with 8 risk factors. The samples from the Department of Obstetrics and Gynaecology gave a precision of 69.9% and 6 risk factors for HSV-, and a precision of 85.3% and 9 risk factors for HSV-2.

DIRECT COMPARISON OF PULSED-FIELD GEL ELECTROPHORESIS (PFGE) AND MULTILOCUS SEQUENCE TYPING (MLST)

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It is thought that Pulsed-Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) have similar discriminatory power but it has been suggested that. MLST is more useful in global epidemiological studies, while PFGE is more sensitive to larger genomic arrangements. In this study, we compare the two methods directly with *Streptococcus pneumoniae*.
PFGE (digestion with *Apa*I) was performed on 30 routine Scottish clinical isolates of serotype 14 with identical MLST type. MLST was performed on 26 Hungarian isolates, of serotypes 6A or 23F, with very similar PFGE patterns. Serotyping was performed with the MAST antisera.

PFGE examination of the 30 serotype 14 isolates with identical MLST type revealed small differences in the banding pattern indicating very little diversity in this group. On the other hand, MLST of the 26 strains with similar PFGE pattern resulted in two main STs (sequence types), i.e. differences in 6 loci out of 7. One ST comprised 12 isolates of serotype 6A with identical PFGE type. Another 11 isolates of serotype 23F belonged to the other main ST. The remaining 3 strains belonged to two further STs. Our results suggest that strains identified as a single MLST type did show some diversity when tested by the more sensitive discriminator of PFGE. MLST determination on strains clustered on PFGE categorised them into sharply distinct groups, indicating that PFGE was better at identifying smaller changes. Interestingly, clustering by both methods showed little diversity within individual serotypes. Different MLST types are easier to identify, as the identification is based on sequences; but PFGE categorisation appeared more discriminatory as it is based on band differences. However, although the two techniques are very different in principle, they are equally important in epidemiology.

TAXONOMICAL DIVERSITY OF SOIL FUNGI IN A CALCAREOUS CHERNOZEM POLLUTED WITH MICROELEMENTS

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Long-term field experiments (LTFE) are fundamental for horticulture and also for the protection of the environment. Ecotoxicology aims to discover chemicals that pose risks in order to apply preventative measures before damage to natural ecosystems occurs. The soil mycota, which represents 30-40% of the total biomass, plays an important role in the mineralisation and other transformation processes in soil. The soil of the experimental site (Nagyhörcsök Experimental Station HAS RISSAC) is loamy calcareous chernozem with 25% clay, developed on loess. In its ploughed layer it contains 3% humus and 3-5% $CaCO_3$. To ensure a sufficient macronutrient supply for the whole experiment, 100 kg/ha N, P_2O_5 and K_2O are given yearly.
The calcareous chernozem soil of the experimental site was rich in fungal populations: 1.09 x 10^6 CFU g-1 and 1.16 x 10^6 CFU g-1 were present in the control plot in July and December 2004, respectively. The huge doses (270 mg kg-1) of both cadmium and selenium resulted in significant and permanent changes in the fungal biomass of the experimental soil. The reduction in CFU was present even 13 years after the treatment. The fungal CFU in the soil treated with cadmium was only 5.48 x 10^5 and 7.36 x 10^5 in July and December, respectively. Similarly, the fungal CFU in the soil treated with selenium was 4.64 x 10^5 and 6.85 x 10^5 in July and December, respectively. The levels of fungal populations both in treated and untreated plots were higher in December than in July.
The effect of microelement treatments on the taxonomical diversity of fungal populations was even more evident: 16 species were obtained from the plots treated with cadmium, 11 from the soil treated with selenium, and 28 from the untreated control soil. 16 of the total of 28 species from the control plot were obtained only from the control soil, with a dominance of the species *Acremoniella atra*, *Cladosporium cladosporoides* and *Scytalidium lignicola*. 10 species of the total of 16 were obtained only from the plots treated with cadmium, with a dominance of the species *Acremonium curvulum*, *A. terricola*, *Cladosporium sp.* and *Fusarium solani*, respectively. Three out of the total 11 species from the plots treated with selenium were cultured only from these samples with a dominance of the species *Aspergillus ochraceus* and *Chaetomium elatum*.
Dependence of diversity upon seasons also increased in the loaded plots: we revealed 9 and 5 constant species from the soils treated with cadmium and selenium, respectively, while 20 in the control

soil. In summary, the quantitative and qualitative ecotoxicological effect of cadmium and selenium can be investigated with testing soil mycota, similarly as testing nematode fauna [1,2].

[1] Bakonyi G et al.: Toxicol Lett **140-141,** 391-401 (2003).
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DEVELOPMENT OF AN EXPRESSION SYSTEM BASED ON A NEWLY ISOLATED METHYLOTROPHIC YEAST

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A methylotrophic yeast strain isolated from a *Quercus* sp. exudate was classified by ribosomal DNA typing as a member of the *Pichia* genus. Two alcohol oxidase genes, *aoxA* and *aoxB*, were identified from the isolate by PCR, using degenerated oligonucleotides designed to be complementary to evolutionarily conserved sequences in the known alcohol oxidase genes. Both *aox* structure genes and the corresponding promoter regions were isolated by one-sided PCR. Methanol induces both alcohol oxidases in *Pichia sp. 159*: the levels of *aoxA* and *aoxB* mRNA reach about 100% and 300%, respectively, of that of *his4* mRNA. *AoxA*, but not *aoxB*, is expressed at a low level in the presence of glucose. The expressions of the two alcohol oxidases were also detected by PAGE and staining, for activity under different growth conditions. Alcohol oxidase activity was detected not only in methanol-grown, but also in starving cells, and in cells growing on a poor carbon source. *AoxA* and *aoxB* promoter-*lacZ* fusions were constructed and their expressions were investigated in a heterologous host. The *aox B* promoter was not active in *Pichia pastoris* growing on methanol, but a substantially delayed expression of *aoxA-lacZ* could be detected. Besides the promoters, other elements necessary for a well functioning expression system were isolated. A detailed analysis of the *Pichia sp. 159* autonomously replicating sequences and the isolation and characterization of the histidinol dehydrogenase gene are presented in the accompanying posters.

STRUCTURAL-FUNCTIONAL ANALYSIS OF [Ni-Fe] HYDROGENASES

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Hydrogenases, catalyzing the following simple reaction: $2H^+ + 2e^- \leftrightarrow H_2$, are harboured by several bacteria, archaea and a few algae. Excess electrons are disposed of from the cell through hydrogen production accomplished by hydrogenases, while consumption of the molecular hydrogen mostly provides electron source for the various energy conserving processes, like respiration. Sometimes hydrogen could be the sole energy source. Hydrogenase enzymes can be distinguished by several properties. Based on the metal content of the active centre of the hydrogenases, they can be classified as Fe-only and [NiFe] enzymes. The [NiFe] hydrogenases are heterodimeric enzymes and the large subunit contains a binuclear metallocenter, in which the nickel and iron atoms are coordinated by cysteinate-sulfur atoms. In addition, three diatomic ligands (two CN- and one CO) bind to the iron. The cysteines coordinating the metals occur in conserved CxxC motifs, which are characteristic for all known [NiFe] hydrogenases. Our model organism was the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina*, contains at least three [NiFe] hydrogenases. The HynSL and the HupSL enzymes are attached to the cell membrane, while HoxYH is localized in the cytoplasm.

We have noticed a highly conserved histidine-rich region with unknown function in the large subunit of hydrogenases. The HxHxxHxxHxH sequence occurs in all membrane-bound hydrogenases, but only three of these conserved histidines are present in the soluble hydrogenases. In order to identify the function of this motif, we used site-directed mutagenesis and started to characterize the biochemical properties of the mutants. The first results showed that enzyme activity was reduced dramatically but still remained in the membrane in the mutant strains. Temperature and oxygen sensitivity of the mutant enzymes are being investigated, proteolytic stability of the enzymes were followed by Western hybridization. A possible role of this basic amino acid stretch is the coordination of the nickel atom, which has been studied by nickel incorporation experiments using 63Ni isotopes.

INHIBITION OF MOULD GROWTH AND AFLATOXIN ACCUMULATION BY FOOD ADDITIVES

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The effect of twelve food additives on the growth of *Aspergillus flavus,* aflatoxin B1 (AFB1) and aflatoxin G1 (AFG1) accumulation was tested at level of 500 ppm. Some of the tested compounds exhibited inhibitory effect on fungal growth and toxin accumulation. Three of investigated compounds, namely 4-hydroxy-3-(p-toluoyl)-6-(p-tolyl)-2H-pyrane-2-one (DHT) and 5-bromo-4-hydroxy-3-(p-toluoyl)-6-(p-tolyl)-2H-pyrane-2-one (BrDHT) and rosemary oil completely suppressed growth and aflatoxin accumulation.
Trials to determine the minimum inhibitory concentration (MIC) of these compounds revealed that DHT was effective at 450 ppm, while BrDHT was highly effective at doses as low as 150 and 100 ppm. In experiments with rosemary oil, the minimum concentration that caused the complete inhibition of mould growth and toxin accumulation was higher than 1000 ppm. It was observed that DHT and BrDHT prevented fungal growth and AFB1 and AFG1 accumulation for up to 14 days. However, after 28 days of incubation, toxin accumulation was greater than in the controls. The MIC values obtained were compared with those determined by the conventional method based on dry matter weight.

A FOLLOW-UP OF MULTIPLE *CHLAMYDIA PNEUMONIAE* INFECTIONS IN A MOUSE MODEL

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Chlamydia pneumoniae is an important cause of respiratory infections, and it has been suggested to be a risk factor in atherosclerosis and immune-reactive chronic inflammatory disorders. Humans commonly encounter *C. pneumoniae*, most individuals having several infections during their lifetime. Animal studies have revealed that *C. pneumoniae* infection in mice causes prolonged pneumonitis, with similarities to the human disease. The significance of the isotypes of antibodies to chlamydial proteins, of the induction of antibodies to bacterial and host heat shock proteins (HSPs), and of the

presence of *C. pneumoniae* DNA in the circulation has not been fully elucidated, and may be of importance in the understanding of the pathomechanism of *C. pneumoniae* infections.
BALB/c mice were inoculated intranasally with *C. pneumoniae* three times to simulate repeated human infections. *C. pneumoniae*-specific antibodies to whole bacterium and antibodies reacting with HSPs were measured by means of ELISA tests. Nested PCR was used to detect *C. pneumoniae* DNA in the peripheral blood.
After primary infection, *C. pneumoniae*-specific IgM antibodies were detected in all animals, and a majority of the mice also demonstrated IgM response after secondary infections. IgG antibodies were detected 2 weeks after the first inoculation, and the titre increased markedly after the second infection. IgA antibodies with high titre were detectable in the sera after repeated infections. Production of antibodies reacting with bacterial HSP60 was induced by infection, furthermore, in the majority of mice, antibodies specific to mammalian HSP60 were detected in high titre after repeated infections. After acute infection, *C. pneumoniae* DNA was readily detectable in the peripheral blood of the mice. The clearance of the DNA was more rapid after the second and third infections, but some mice gave a positive PCR test at later time points, even after a single inoculation.
Analysis of the occurrence of immunoglobulin isotypes in the serum after multiple *C. pneumoniae* infection may provide a clue to the evaluation of diagnostic serological tests. *C. pneumoniae* reinfections result in the production of antibodies that react with bacterial and endogenous HSPs with potential roles in protection against infection and in the development of autoimmune mechanisms. The presence of *C. pneumoniae* DNA in the blood can serve as an additional tool for the diagnosis of acute and persistent infection, and for epidemiological studies in which the contribution of infection to atherosclerotic diseases is assessed.
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ENZYMATIC PRODUCTION OF CHIRAL 1-ARYL- AND 1-ARALKYLETHANOLS

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Chiral 1-aryl- and 1-aralkylethanols are frequently used building blocks in the synthesis of novel pharmaceuticals and fine chemicals. Optically clear alcohols can be efficiently achieved by two enzymatic ways: (1) from prochiral ketones with enantioselective reduction by carbonyl reductases or (2) from *rac* alcohols with stereoselective esterification catalysed by lipases. Oxidoreductases require regenerated cofactor to reduce prochiral ketones. The whole yeast cell biocatalyst reduced the ketones and, in the presence of the co-substrate, they were capable of regenerating the required form of the cofactor (NAD(P)H). Biocatalysts work under mild and safe conditions.
Eight yeast strains known of their reductase activities were monitored by reduction of phenyl- and benzylacetones. Two strains were chosen to examine their substrate tolerance on 11 aryl- and aralkylmethyl ketones. Good yields were obtained except for the 2-substituted methoxy derivatives. The ee values of the produced alcohols were also monitored. The transport of the cell paste is difficult, furthermore, its storage has not yet been solved. A lyophilization method was established, which resulted in a conveniently moveable yeast powder having a similar reductase activity to the fresh cell paste. One known carbonyl reductase of *Z. rouxii* was cloned and expressed in *E. coli.* The results of the bioreductions obtained with the recombinant gene carrying *E. coli* were similar to that obtained

with Z. rouxii, except when trying to reduce benzylacetone and acetophenone. Shorter (acetophenone) and longer (benzylacetone) carbon chain containing ketones were not substrates of the expressed enzyme. This result proved that *Z. rouxii* has more than one carbonyl reductase enzyme.

COMPARING OBSERVED GROWTH OF SELECTED TEST ORGANISMS IN FOOD IRRADIATION STUDIES WITH GROWTH PREDICTIONS CALCULATED BY COMBASE SOFTWARE

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To ensure the safety of minimally processed refrigerated foods, we need to know if specific pathogens could grow, and, if so, how quickly. The use of microbial models will play a key role in the scientific foundation of the Hazard Analysis Critical Control Point approach in new food processes. As a result of intensive predictive microbiological modelling activities, several computer programmes and software became recently available for facilitating microbiological risk assessment. Among these tools, the establishment of ComBase, an international database and its predictive modelling software of the Pathogen Modelling Program (PMP) set up by the USDA Eastern Regional Research Center (Wyndmore, PA) and the Food Micromodel/Growth Predictor by the UK MAFF Food Research Institute (Norwich) are most important.
We used the PMP 6.0 software version of ComBase as a preliminary trial to compare observed growth of selected test organisms, in relation to our food irradiation work during the recent years within the FAO/IAEA Coordinated Food Irradiation Research Projects D61023 and D62007, with their predicted growth on the basis of growth models, are available in ComBase for the same species as our test organisms. Results of challenge tests with *Listeria monocytogenes* inoculum in untreated or irradiated experimental batches of a semi-prepared breaded turkey meat steaks („Cordon Bleu"), sliced tomatoes, sliced watermelon, sliced cantaloupe, and sous-vide processed mixed vegetables, as well as *Staphylococcus aureus* inoculum of a pasta product, Tortellini, were compared with their respective growth models under relevant environmental conditions. This comparison showed good fits in case of non-irradiated and high moisture food samples, whereas growth of radiation survivors lagged behind the predicted values.

COMPARISON OF TRADITIONAL AND MOLECULAR MICROBIOLOGICAL METHODS FOR THE DETECTION OF *SALMONELLA*

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Salmonella serovars are of great epidemiological significance as the major cause of human gastrointestinal and extraintestinal infections. The primary sources of salmonellosis are predominantly poultry, pork and beef, therefore it has been crucial for a long time to monitor and control the occurrence/prevalence of these pathogens in the food chain, "from farm to fork".

The standard detection method of *Salmonella* relies on conventional culture-based techniques, consisting of enrichment of samples in liquid broth, incubation on selective agar plates, finally subspecies determination/serotyping of the *Salmonella* candidates in a central reference laboratory. The current standard laboratory procedure to culture and identify *Salmonella* serovars takes approximately 4-7 days. Molecular microbiological methods offer a rapid, sensitive and cost-effective alternative to traditional approaches: different PCR assays, in general, are more universally available for the detection/serotyping of *Salmonella*, however, due to the lack of international validation and standard protocols, the application of PCR for diagnostic use is yet to be solved.
Traditional and molecular microbiological techniques were compared in our laboratory regarding sensitivity, specificity and the conformity of results. The standard culture-based diagnostic method involved enrichment in Buffered Peptone Water and Rappaport-Vassiliadis broth, and plating onto xylose-lysine-tergitol 4 agar (XLT-4). Serotyping was carried out in a reference laboratory. Molecular methods consisted of extracting DNA from the sample with/without an enrichment step and detection of the pathogen by PCR assays, one targeting the invA gene located on the *Salmonella* pathogenicity island 1 (SPI1), the other specific for a gene coding a major outer membrane porin protein (ompC). A commercially available real-time PCR was also tested using DNA purified from different sources (faeces, colony etc.).
As a result, a rapid test was developed for the detection of *Salmonella*:
- DNA is directly extracted from sample material (carcass rinse, meat, faeces, colony, etc.)
- (real-time)-PCR is performed to detect *Salmonella* specific genetic material.

Results are obtained within 5-6 hours upon the arrival of sample material. A multiplex PCR is under development to identify the most common agents of foodborne infections in Hungary: *Salmonella enteritidis*, *S. infantis*, *S. typhimurium*.

DEVELOPMENT OF GENETICALLY ENGINEERED NUCLEIC ACID VACCINES FOR IMPROVED PROTECTION OF CATS AGAINST FELINE INFECTIOUS PERITONITIS

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Feline infectious peritonitis (FIP) is currently the leading infectious cause of death among young domestic and wild *Felidae*. The disease has a worldwide distribution, especially in cats originating from purebred catteries. The causative agent of FIP is feline coronavirus (FCoV) belonging to the Coronavirdae family. The incidence of FIP in a cat population is correlated with the FECV $\rightarrow$ FIPV mutation rate, in which the ORF7 gene may play a role. Both the diagnosis and control of the disease is rather complicated. At present, no curative treatment against FIP is available, however, the prognosis for a cat having FIP is very poor. A commercially available vaccine has given promising results only in FCoV naive but not in FCoV carrier cats. The humoral immunity is not protective against FIP. The activation of the cellular immune response may offer a better result. In case of Infectious Bronchitis Virus of chicken, the nucleocapsid (N) gene was proved to be a good inducer of cellular immunity, for this reason in our experiments the N and ORF7 genes had a special interest.
The nucleocapsid and the ORF7b genes of the DF-2 strain of feline coronavirus (FCoV) were amplified by polymerase chain reaction (PCR). The amplified products were cloned into the pCI expression vector, which lacks the neomycin resistance gene. Nucleotide sequencing of the recombinant plasmids proved that the viral genes were situated in the right position and orientation. The following constructs were generated: i.) pcI expression vector containing nucleocapsid gene of FCoV (Con-

struct 1); ii.) pcI expression vector containing ORF7b gene of FCoV (Construct 2). Construct 1 was used in model and host animal experiments. In the model animal experiment, 50 BALB/C mice were inoculated intramuscularly with 1, 50, 250 mg of Construct 1. The inoculations were carried out with 2-week intervals. Blood samples of of mice were tested by cell proliferation assay. In the host animal experiment, 11 cats, 14-16 weeks of age, were obtained from a commercial supplier, and were previously tested for FCoV by ELISA and diagnostic RT-PCR. Nine animals were vaccinated i.m. and challenged intranasally with DF2 strain and the remaining two were used as controls. During the 90-day observation period, coagulation inhibited blood samples were collected twice a week and were tested by FACS.

Both Constructs were proved efficient in vitro by RT-PCR and immunofluorescence assay. Construct 1 was chosen for in vivo evaluation, because its putative in vivo efficacy was supported by the results of other research groups. Construct 1 has a concentration-dependent effect on the proliferation of lymphocytes in mice. Host animal experiment has not given proper protection against challenge, however, the life of the challenged animals was elongated. Cellular immune response seems to be active, however it was not sufficient for survival. The present study has added new data to our knowledge regarding the immune mechanism happening during FIP infection.

SUSCEPTIBILITY TESTS IN MICE AND RATS TO CLASSICAL SWINE FEVER VIRUS

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Classical swine fever (CSF) is a highly contagious viral disease of pigs known for more than 150 years, considered by the World Organisation for Animal Health (OIE) as list A disease because of its vast potential to cause serious consequences for the trade of pigs and pig products. The causative virus (CSFV) is the member of genus *Flavivirus*, family Flaviviridae together with BVDV-1, BVDV-2, BDV and H138, which is a virus isolated from giraffe. The members of the family Flaviviridae show strong antigenic and structural relations. Previously, Pestiviruses were classified by host-specificity, but the advent of the genetic diagnostic methods revealed that Pestiviruses can be spread from sheep to cattle or from ruminants to pigs. Altough the CSFV is considered as a Pestivirus with the most restricted host-range, pets and rodents are frequently suspected as possible virus transmitters of CSFV, because many times secondary infections are observed in the vicinity of the large CSF outbreaks. To clarify the potential active role of rodents, mice and rats were infected experimentally.

In our experiments, 40 mice were used, divided into two groups each containing 15 mice. Group I was infected orally, while infective virus was applied intramuscularly in group II. Ten mice were kept separately as controls, five to group I and five to group II. A group of 10 rats was infected intramuscularly and 5 rats were used as controls kept separately. During the 4-week long observation period, faeces, plant and coagulation-inhibited blood were taken, and after euthanasia organ specimens were collected from the brain, lung, spleen and intestine. All animal were subjected to gross pathological examination. The blood samples were examined by commercial CSFV Ab ELISA Kit. RNA was isolated by TRIzolTM from organ specimens and blood samples, and PCR assays were carried out.

No clinical signs related to CSFV experimental infection were found. No macroscopic changes were observed during the pathological examination. Neither orally infected nor intramuscularly infected animals contained antibodies against CSFV. All PCR were negative in organ specimens and blood samples. Several reasons may support the concept of the potential transmitter role of rodents. Despite the restricted host range, CSFV was adapted to rabbits (lapinised strains), moreover, the inactivated

vaccines containing CSFV-related BVDV strains can be evaluated during the standard potency tests of vaccine control. Considering these facts altogether, the potential role of mice and rats as animals living in the proximity of pigs had to be clarified in order to obtain a better picture of their role in CSFV transmission. Our results indicate that neither mice nor rats are susceptible to CSFV infection. For this reason, their active role in virus transmission can be ruled out, however, the passive transmitter role remained as a possibility. The likelihood of mechanical transmission is difficult to assess and was beyond the objectives of this experiment.

CHARACTERIZATION OF THE EXPRESSION OF A GENE ENCODING A LACTOSE PERMEASE IN THE FUNGUS *ASPERGILLUS NIDULANS*

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Lactose metabolism of the filamentous Ascomycota fungus *Aspergillus nidulans* is a dominantly intracellular process. Lactose is transported by a specific permease, thereby inducing the activity of the intracellular beta-galactosidase(s), which, subsequently, hydrolyse it into glucose and galactose. No report has been published to date on the characterization of a fungal lactose permease. Addition of glucose into lactose-grown wild-type *A. nidulans* cultures blocked further lactose uptake. However, when the glucose pulsing experiment was repeated with a mutant strain in which the creA locus, responsible for the CreA-dependent carbon catabolite repression had been deleted, lactose consumption remained unaffected, thereby resembling the non-pulsed control culture. Thus, the effect of glucose on lactose uptake is apparently due to CreA-dependent carbon catabolite repression. To investigate this possibility in more detail, mycelia of the wild-type as well as the creA mutant strain were pregrown in glycerol and were transferred into a fresh medium with 1 % lactose as a sole carbon source. Lactose uptake was monitored by the disappearance of the 14C labelled form. Within 3-4 hours, addition of glucose halted further lactose uptake in the wild-type, but not in the creA mutant strain, in which no changes in the lactose uptake rate was observed. The non-repressing carbon source L-arabinose, on the other hand, exerted no effect on either *A. nidulans* strain. This result further underlines the relationship between lactose permease and the carbon repressor CreA, which, judged by the several hour-long period before the effect actually occurred, most likely proceeds on the level of transcription. Interference of CreA with permeases (such as here with the lactose permease) has not yet been shown. To understand this relationship at molecular level, an *A. nidulans* gene of 790 bp was isolated upon homology to the yeast *Kluyveromyces lactis* lactose permease gene, was amplified by PCR and used as a Northern hybridization probe. Comparative Northern analysis of this lacp1 gene in the wild-type as well as in the creA mutant strain indicated that the expression of lactose permease is regulated by the carbon source, and, most importantly, is completely repressed by glucose in a CreA-dependent way.

MOLECULAR ANALYSIS OF THE DIVERSITY OF PICOPLANKTON POPULATIONS IN SOME HUNGARIAN FRESHWATERS

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Photoautotrophic picoplankton is a widespread functional group of bacterial sized organisms in water, which can provide as much as 90 percent of primary production in the oceans. These organisms are also abundant in Hungarian shallow lakes, moreover, lakes having high salt concentrations are extraordinarily rich in planktonic picocyanobacteria and picoeucaryotes. Since taxonomic investigations are not possible with conventional analysis because of the small cell size, limited number of distinct morphological features and known difficulties of cultivation, the species composition of picoplankton can mainly be examined by molecular methods. The survey of some Hungarian lakes (Lake Balaton, Zab-szék, Böddi-szék, Büdös-szék, Kelemen- szék, etc.), with picoplankton dominance in primary production, concerning the identification of the dominant members of the picoplankton community was performed according to the following:
- determination of the species composition (based on total DNA extraction, cloning, sequence and phylogenetic analysis of the clones),
- elaboration and optimization of a cost-saving molecular fingerprint method (DGGE: denaturing gradient gel electrophoresis) applied in diversity analysis,
- application of these methods to perform investigations focused on seasonal dynamics.

Our sequences showed high similarity values to both phycoerythrin- and phycocyanin-rich *Synechococcus* isolates derived from different environments (oligo-mesotrophic subalpine lakes, Bornholm Sea and Lake Biwa, Japan), as well as to *Chlorella* (eukaryotic green algae) plastid sequences.

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AMINO ACIDS MODULATE THE ANTIPROLIFERATIVE ACTIVITY OF HUIFN-ALFA AGAINST MALIGNANT CELLS IN VITRO

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Natural HuIFN-a represents a heterologous mixture of different subtypes with the more or less predominant antiviral, antiproliferative, antitumor, cytocidal, immunomodulatory , radioprotective and various other activities. It was previously found that certain amino acids can modulate (increase and/or decrease) the antiviral (AV) activity of HuIFN-a in vitro. The experiments presented here were aimed to find out if the amino acids influencing the AV activity of HuIFN-a can also modulate the antiproliferative (AP) activity of natural HuIFN-a. Several natural HuIFN-a preparations from the Institute of Immunology (Zagreb, Croatia) and EGIS Pharmaceutical Factory (Budapest, Hungary) were used. In the experiments the following amino acids were used: L-asparagine, L-aspartic acid, L-cysteine, L-serine, and DL-theronine. Amino acid stock solutions were prepared in 2N HCl at a concentration of 10mg/ml. The influence of amino-acids on the AP activity was measured on two malignant cell lines: CaCo-2 (Colon cancer carcinoma) and Rudi.-1 (Leukemic macrophage cell line). Cells were cultivated in Eagle's Minimal Essential Medium (EMEM) supplemented with 8% of SR-2.055P. The AP activity was obtained by the growth index (GI) inhibition assay performed on Caco-2

and Rudi-1 cells. Mixtures (1:1 ratio) of IFNs in a concentration of 10 and 1000 IU/ml and different amino acids in a concentration of 1mg/ml were added to the cell cultures.
The results show that L-asparagine and L-serine were the most effective in enhancing the AP activity of the different preparations of natural HuIFN-a, followed by DL-theroneine, L-aspartic acid and L-cystein in decreasing order of influence. The observation is also noteworthy that the different AV:AP ratios obtained with the various preparations of natural HuIFN-a could be modulated by applying different amino acids concomitantly with the HuIFN-a preparations.

THE BIOLOGICAL ASSAYS OF INTERFERONS

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Even more than a quarter of a century has passed since the discovery of Interferons (IFN), still a universally accepted method for their biological assay is still not generally approved. One of the reasons lies definitely in the fact that IFNs are pleiotropic molecules showing not only antiviral but also antiproliferative, cytocidal, antitumor, immunomodulatory and radioprotective activities. At present, the most widely used method for IFN quantitation is based on the measurement of various parameters of viral replication in IFN-treated cells. Among these parameters, the one based on cytophatic effect (CPE)-inhibition offers the most useful assay to determine the biological activity of IFN. Such an activity is expressed in I.U. (International units)/ml. Although the diploid fibroblast cells can be more sensitive, the use of different continuous cell lines is more practical. The purpose of this study was to compare three established cell-lines (WISH,HAC-3/T2 and MDBK) to ascertain which cell-virus is more appropriate for quantitation of both Human and Porcine Interferons (Alpha, Beta and Gamma). Subsequently, the antiviral activity was compared with the antiproliferative with the same cells and cytocide using the PLA-1 (Adult pig kidney cell line) cells. The sensitivity and reproducibility of the different test systems were also determined, eventually leading to the biological activity of IFNs.

COMPARATIVE PATHOGENESIS OF MOUSE-ADAPTED INFLUENZA VIRUSES OF DIFFERENT VIRULENCE

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Three strains of influenza virus, namely A/Dunedin/4/73 (H3N2), A/Mississippi/1/85 (H3N2) and PR/8/34 (H1N1) were adapted to BALB/c mice. Their infectivity on MDCK cells (TCID50), their virulence for mice (LD50) and the minimal infectious dose for mice (MID) were determined. We did not find any significant difference among viruses examined in their infectivity *in vitro*. However, the following differences in virulence were observed: while 1LD50 of Dunedin strain corresponded to 2.8 HU, 1LD50 of the Mississippi strain represented 0.096HU and that of PR/8 contained 0.00128 HU only. Therefore, the virulence ratios for Dunedin/Mississippi/PR/8 strains were 1:29:2175, the latter virus being about 2000 times more pathogenic than the Dunedin virus. The MID value for Dunedin was 1/3500 LD50, that for Mississippi was 1/7500 LD50 and that for PR/8 was 1/4476

LD50. Thus, the ratios of LD50/MID among the given strains did not differ significantly and remained constant unlike their virulence. Determination of serum antibody titers after i.n. immunization of mice using the viruses in question showed comparable values. Histological and immunohistological investigations of the lungs of infected mice revealed that the Dunedin strain caused significant bronchitis and peribronchitis with extensive staining of viral nucleoprotein (NP) antigen in the nuclei of many bronchiolar cylindrical epithelium cells, which appeared positive already from day 2 post-infection (p.i.). The Mississippi virus induced bronchitis accompanied with more extensive round cell infiltration of alveolar septa seen mainly on day 4 p.i.; the viral NP antigen was present in the cylindric epithelium cells as well as in a few alveolar macrophages. Infection with the PR/8 virus was associated with severe bronchopneumonia, characterized by focal mononuclear exudation to alveoli and to the lumen of bronchi since day 2 p.i. The nucleoprotein antigen was harbored in very few scattered bronchial epithelium cells and was rather abundant in groups of macrophages. Following latter infection, the paucity of alveolar epithelium involvement contrasted with the extensive bronchopneumonia, which might be the reason of high pathogenicity of the PR/8 strain.

FUNCTIONAL ANALYSIS OF *ALC*S, A GENE OF THE ETHANOL UTILIZATION (*ALC*) CLUSTER IN *ASPERGILLUS NIDULANS*

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The saprophytic hyphal fungus *Aspergillus nidulans* is a highly versatile microorganism capable of growing on ethanol as the sole source of carbon. Ethanol utilization requires two structural genes, *alcA* and *aldA*, encoding the dehydrogenases responsible for the conversion of ethanol into acetate *via* acetaldehyde, and a pathway-specific regulatory gene encoding the transcriptional activator AlcR. The *alcR* and *alcA* genes are clustered with three other genes that are also positively regulated by *alcR*. The *alcS* gene is the most abundantly transcribed of these three genes of unknown function. It is strictly co-regulated with *alcA* and encodes a 262-amino acid protein. Database comparisons revealed an amino acid sequence motive in AlcS, characteristic of the GPR1/FUN34/YaaH family of membrane proteins and indeed, the protein was found specifically located in the plasma membrane. However, deletion or overexpression of *alcS* did not result in any obvious phenotype. Moreover, AlcS does not appear to be involved in the transport of ethanol or that of acetaldehyde or acetate, the first two intermediates of ethanol catabolism, over the plasma membrane. Comparative analysis of the *A. nidulans* genome sequence led to the identification of two other genes encoding putative transmembrane proteins of the same family that are co-induced with *alcS*. This mutual expression of structurally related proteins suggests a functional redundancy that could explain why the *alcS* gene (unlike *alcA*, *aldA* and *alcR*) is dispensable for ethanol utilization.

PRESENCE AND PHYLOGENETIC RELATIONSHIP OF HEPATITIS E VIRUS OF ANIMAL ORIGIN IN HUNGARY

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Hepatitis E Virus (HEV) is a non-enveloped, positive-sense, single-stranded RNA virus with icosahedral symmetry, belonging to the genus *Hepevirus* in the family *Hepeviridae*. HEV is enterically transmitted and the infection in humans is associated mainly with an acute, self-limited, icteric hepatitis and with a mortality of 1% (up to 20% in pregnant women). Antibodies to HEV were found in different animal species; however, the HEV infection was asymptomatic. The HEV strains isolated from animals are genetically related to human HEV strains; therefore zoonotic transmission is suggested. Besides imported cases of hepatitis E from endemic developing countries with more than 100 HEV infections per year in Asia and Central-America, domestically acquired hepatitis E has been reported recently in many "non-endemic" industrialized countries including the USA, European countries, Japan and New Zealand, and HEV antibodies have also been found up to 3.3% of these populations.

Recently, a survey has started in Hungary to reveal the presence of HEV in domesticated and wild animals potentially subjected to human consumption. Between January and April 2005, 157 samples of different animal origin have been tested for HEV RNA by reverse transcription-polymerase chain reaction (RT-PCR) with primers targeting the ORF2 region. HEV RNA was detected in liver samples of wild boars (8 out of 58 samples), roe-deers (8 out of 22 samples), deers (3 out of 9 samples), and both liver and faeces samples of domestic swine (10 out of 14 farms) in different regions of the country. By sequencing of the PCR products and phylogenetic analysis of the sequences, HEV genotype III strains were identified and found to be related to other HEV strains of human and swine origin.

The results show for first time that HEV is present in both domesticated and wild animals in Hungary.

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DEMONSTRATION OF CHRONIC PARALYSIS VIRUS OF HONEY BEES USING RT-PCR AND AN ELECTRON MICROSCOPIC SURVEY OF THE CAUSATIVE AGENT

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At least 18 viruses have been reported to infect honey bees worldwide. Some of these have been detected also in Hungarian samples, including Kashmir bee virus (KBV), acute bee paralysis virus (ABPV), sacbrood virus (SBV), black queen cell virus (BQCV) and deformed wing virus (DWV). Though based on the clinical symptoms the occurrence of the chronic bee paralysis virus (CBPV) was also suspected, it has not been proven so far. On the other hand, due to the similarity of the clinical signs characterizing chronic paralysis of honey bees with other symptoms caused by other infectious and non-infectious etiological agents (acute paralysis, pesticide intoxications, exhaustion of the bee colonies, etc), it is very important to develop reliable diagnostic methods to prove the presence of the virus in diagnostic samples. The possibilities of conventional investigations are rather limited in case of bees, thus the recent advances in molecular diagnostic techniques have enhanced the chances of detection and analysis of honey bee viruses. The techniques applying polymerase chain reaction

(PCR) are fast and reliable, and combined with other methods of investigation, such as electron microscopy, are powerful aids in identifying viruses and determining their accurate taxonomy and their phylogenetical relationship to other viruses. The importance of the electron microscopic survey is even higher, because while the other major viruses of the honey bee have similar morphology (icosahedral capsid) and are of approximately the same size, CBPV has no definite capsid structure (pleomorphic) and its diameter varies from 30 to 45 nm.
A primer pair specific for and designed using the only known segment of the CBPV genome was applied for diagnostic purposes. Samples from a Hungarian bee colony have been positively tested for CBPV following successful nucleic acid purification. After the definite diagnosis, the samples were examined with electron microscope and pictures of ellipsoidal viral particles, of about 30-45 nm in length and approximately 20 nm wide, were taken. Despite its wide distribution the taxonomy of this virus is still not clarified, it could not have been characterized well enough to be assigned to any of the virus families known so far. The new results confirm the presence of the virus for the first time in Hungary. Further investigations are planned to determine its exact taxonomy and relationships with other viruses.

CLINICAL ASPECTS AND PHARMACOLOGICAL CONSIDERATIONS IN ANTIMICROBIAL THERAPY OF BACTERIAL COMPLICATIONS OF POSTPARTUM UTERINE INVOLUTION IN DAIRY COWS

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Uterine involution in cows is by far not a sterile process even in physiological situations. In the *first two weeks* of postpartum, a wide variety of bacteria can be isolated from both the uterus of cows with normal involution (healthy cows), as well as of those showing signs of bacterial complications, such as the acute putrid form of puerperal (endo)metritis (APE) at that time. However, significant differences are demonstrated considering the frequency distribution of bacteria: while *Staphylococci*, *Streptococci*, *Bacillus spp.* and (non *E.coli*) *coliform bacteria* were predominant in the uterine swab samples from healthy cows, the frequency of *Arcanobacterium pyogenes*, *Escherichia coli* and some Gram-negative anaerobes (*Fusobacterium necrophorum, Bacteroides spp.*) were significantly higher in those with APE. Moreover, *A. pyogenes* often can be isolated along with Gram-negative anaerobes. These bacteria are, therefore, so called “uterine pathogens”. At the *later postpartum period* (>14 days pp), however, healthy uterus gradually eliminates all bacteria by its natural self-defence mechanisms. In the cases of chronic, (muco)purulent forms of endometritis (Chr EM) or pyometra, *E. coli* is much less frequent than in APE, and *A. pyogenes* and Gram-negative anaerobes are the predominant pathogens. Presence of these bacteria in the uterus at 28-35 days post partum has an obvious negative effect on reproductive performance, significantly decreasing the chance for re-conception. From a therapeutic point of view, the obvious target is to eliminate “uterine pathogens”, therefore an efficacious antibacterial treatment (among others) is essential. A good product for intrauterine use must contain active ingredient(s) (i) with low MIC for all important uterine pathogens; (ii) resistant to β-lactamase, because bile resistant *Bacteroides* spp. produce this enzyme; (iii) able to act under extreme (highly anaerobic, rich in necrotic debris and pus), conditions of post partum endometrium. Vehicle to ensure proper distribution of the product is also of great importance. Parenteral injectable antibiot-

ics are also a matter of choice, however, milk (and tissue) residue is the crucial point, since dairy cow is a food-producing animal. By using injectable antibiotics, the beneficial effect of uterus manipulation may also be lost. Some years ago, the therapeutical aspects of APE and Chr EM were studied in 4 series of clinical trials to evaluate the relationship between the clinical picture and results of bacteriological examinations, and to assess the efficacy of different therapeutic protocols based on the use of some antimicrobials (APE: oxytetracyclin, amoxicillin, gentamycin; Chr EM: cephapirine, penicillin+neomycin, tylosin, inorganic iodine) and/or prostaglandin F2a (PGF2a). Therapy was effective when it enhanced the elimination of *A. pyogenes* and Gram-negative anaerobes from the uterus.

BIODEGRADATION OF OIL-CONTAMINATED SOILS BY MICROBIAL COMMUNITIES

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Crude oil and its refined products are widely used in the industry and in everyday life. As a consequence of careless handling, a singificant amount of oil and its derivatives is released into the environment. Oil can be removed from the environment by mechanical, chemical and biological methods. It is relatively easy to collect from the surface of water, however, the remediation of contaminated soils is more sophisticated. Physical-chemical methods are generally applied for highly polluted areas, while the biotechnological approach is used for less contaminated fields.

Numerous bacteria capable of degrading aliphatic and aromatic hydrocarbons have been isolated. Because oil is water insoluble these microbes usually produce surfactants for making oil accessible for the biochemical processes. In the most efficient biodegradation methods, oil is oxidized by specialized oxygenases, therefore oxygenation of the system is required. For this reason, soil is usually arranged into a special shape, named prism, where the depth of the polluted soil is 30-50 cm.

A bacterium capable of degrading hydrocarbons has been isolated in our laboratory. The strain was taxonomically characterized and identified as *Rhodococcus erythropolis* MK1.

Soil sample contaminated by heavy-, and fuel oil was obtained from Mohács (7000 mg TPH/kg soil). Our aim was to develop a technology for the remediation by an ex-situ on site approach using bioaugmentation combined with biostimulation.

Our *Rhodococcus erythropolis* MK1 isolate was cultivated in a 50 l fermenter and then spread onto the soil mixed with chopped straw and collected to prisms. Soil samples were taken monthly during the treatment and the microflora content of the samples was investigated. At the end of the treatment (three months) the total hydrocarbon concentration of the samples was reduced below 300 mg TPH/kg soil which was less than the threshold value. Since the pollutants could not be removed by the indigenous community, it was concluded that our isolate had a substantial contribution to this process. It is noteworthy that although *R. erythropolis* MK1 might have been involved in the direct biodegradation process, it probably also had a biostimulative effect. Indeed, the composition of the microbial community in the soil samples was altered according to the actual TPH content and composition of soil, nevertheless our isolate was present approximately at the same level.

INCIDENCE AND ANTIBIOTIC RESISTANCE OF MAJOR BACTERIAL PATHOGENS IN HUNGARY IN 2004

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Data collected on the incidence and antibiotic resistance profile of major bacterial pathogens by the „Hungarian Microbiological Surveillance System" in 2004 is presented. Some pathogens /MRSA, penicillin-resistant *Streptococcus pneumoniae*/ show characteristic geographical distribution in Hungary, while others /*Pseudomonas aeruginosa*, SHV-type ESBL-producing *Klebsiella pneumoniae*/ affect patients mainly in particular hospital wards. Rates of antibiotic resistance have significantly increased for several pathogens in recent years. The proportion of MRSA rose to 17.3% among invasive isolates of *Staphylococcus aureus* in 2004. The rate of macrolide resistance in *Streptococcus pneumoniae* reached 33.7% in hospitalized patients and 17.0% of invasive *Pseudomonas aeruginosa* isolates proved resistant to imipenem. The greatest rise was detected in the resistance rate of *Klebsiella pneumoniae* to ceftazidime and cefotaxime reflecting mainly the dissemination of ESBL-producing nosocomial pathogens across the country. In addition to identifying major shifts in resistance rates, comprehensive data is provided on all important agents and antimicrobials.

HOW THE POTENTIAL ANTIFUNGAL DRUG ENTERS THE CELLS OF *CANDIDA ALBICANS*

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Icofungipen is an antifungal agent of the beta-amino acid class. It has a dual mode of action on the susceptible fungi. The dual mode of action is based on an active accumulation of icofungipen in the cell of *C. albicans* and inhibition of protein synthesis due to the inhibition of enzymatic activity of isoleucyl-tRNA synthetase.

Icofungipen as b-amino acid enters the cell probably via an active amino acid transport of *C. albicans*. There are several amino acid transporters in *C. albicans* that might be involved in the active transport of icofungipen into the cell. Data obtained so far indicate that icofungipen is transported into the cell via branched chain amino acid transporter(s). To test this hypothesis, changes in MIC values of icofungipen, after addition of amino acids in the test medium, were tested in strain *C. albicans* ATCC 90028. MIC values of icofungipen decreased when the YNG medium was supplemented with the amino acids: arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, histidine, lysine and serine. In contrast to that, branched chain amino acids such as isoleucin, leucin and valine, as well as phenylalanin and tryptophane significantly increased MICs of icofungipen, when added to the YNG media in the same strain of *C. albicans*. We can conclude from the results obtained that charged amino acids have a positive influence on icofungipen activity *in vitro* on tested *Candida albicans* strain when added to the YNG medium.

TOPO-OPTICAL INVESTIGATIONS ON THE CELL WALL OF VARIOUS YEASTS

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The aim of this study was to selectively analyse the structure of sugar components in the cell wall of various yeasts. Romhányi's topo-optical staining reactions (Romhányi et al., 1975) are suitable for submicroscopic research of the cell wall of various fungi. After applying the Aldehyde-Bisulphite-Toluidine blue-reaction (ABT-r) to different *Candida* strains the optical analysis showed differences in the orientation of sugar molecules. *C. albicans* and *C. krusei* produced linearly positive (radially negative) anisotropy under the polarisation microscope. Sugar components of *C. glabrata* and *C. tropicalis* were orientated the opposite way (linearly negative, radially positive).
Further research revealed an influence of the culture medium on the orientation of sugar moieties. Using Yeast extract peptone dextrose agar medium, sugar components were visible when applying the topo-optical reaction after 24 hours. A positive staining reaction on three fungal species (*C. albicans*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae*) was seen with 0.1% aqueous Congo red solution. The anisotropy is linearly positive (radially negative).
It is interesting that *C. albicans*, *C. neoformans* and *S. cerevisiae* do not show an anisotropic effect with a 1% aqueous solution of Eosin, but do so after a pre-treatment with Chlorpromazine. This is a charge transfer reaction. All three species produce a linearly positive (radially negative) reaction. Furthermore, the outer layer of *C. neoformans* contains sugar moieties in the opposite orientation, too.
The sialic acid components in the cell walls of *C. albicans*, *C. neoformans* and *S. cerevisiae* are selectively demonstrated by using a KOH sialic acid specific topo-optical reaction. This reaction indicates that *C. albicans* has sialic acids in a linearly negative (radially positive) orientation. *C. neoformans* has O-acyl sialic acids in the outer layer (orientated linearly positive, radially negative) as well as in the inner layer (orientated linearly negative, radially positive). *S. cerevisiae* has sialic acids and O-acyl sialic acids orientated linearly negative (radially positive).

SELF-DEFENDING BIODEGRADATION OF TOXIC COMPOUNDS: COUPLING THE UPTAKE AND OXIDATION OF SULFANILIC ACID IN *SPHINGOMONAS SUBARCTICA*

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Sphingomonas subarctica SA1, a Gram-negative aerob bacterium was isolated in our lab. The strain is solely able to utilize sulfanilic acid as the only carbon, nitrogen, and sulfur source. In addition to sulfanilic acid, our isolate was able to degrade six other aromatic compounds, like sulfocatechol, protocatechol, para-amino benzoic acid, 3,5-dihydroxy-benzoic acid and oil in soils. Comparison of the protein patterns of cells grown on different substrates revealed that the strain used alternative metabolic pathways for biodegradation of the various compounds.
Sulfanilic acid could only be converted by intact cells (not by disrupted cells) and supposedly, sulfocatechol is formed in this reaction. Sulfocatechol is further oxidized by a ring cleaving dioxygenase, named as sulfocatechol dioxygenase. This enzyme was partially purified and identified by mass spectrometry. A genomic locus harbouring the genes of sulfocatechol dioxygenase (*scaDE*) was also identified and upstream from these genes, few other *orfs* coding for proteins similar to muconate cycloisomerases (ScaA), lactone hydrolases (ScaB) and maleilacetate reductases (ScaC) were recognized. These enzymes were actively overexpressed in *E. coli* and the sulfocatechol degradation pathway was reconstituted by the recombinant proteins.

The enzyme, probably hydroxylating sulfanilic acid, was very sensitive to cell disruption indicating that the enzyme catalyzing this reaction was somehow related to the membrane. The proteomics approach was applied to identify the enzyme(s) catalyzing the sulfanilic acid conversion. Bands specifically appeared upon substrate induction in the membrane, and soluble fractions were cut out and sequenced *de novo* by mass spectrometry. Two proteins were identified in both fractions: a glutamine synthase type protein, probably responsible for removing the amino group, and a conserved oxidoreductase with unknown function. Additionally, peptides characteristic for ATP binding proteins could also be identified in the membrane fraction suggesting a connection to an energy dependent – probably transport – process. The gene for the glutamine synthase was isolated and sequenced and an *orf* coding for a putative ring hydroxylating dioxygenase was discerned in its vicinity. The gene of the conserved oxidoreductase was found in the gene cluster of the sulfocatechol dioxygenase (*sca*) locus, indicating a functional relationship among the gene products.
From these data, it is assumed that the enzyme catalyzing the oxidative hydroxylation of sulfanilic acid is loosely membrane associated. On the other hand, preliminary data suggest that the transport of this compound may be an active energy requiring process. It might be speculated that a tight link between these processes could provide a self-defending mechanism for the cell against the cytoplasmic occurrence of the toxic sulfanilic acid. Detection of protein-protein interactions would provide more direct evidence to support this working hypothesis.

INDUCTION OF HEAT TOLERANCE IN *CLOSTRIDIUM PERFRINGENS* BY EXTRACELLULAR COMPOUNDS

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Several reports have shown that at supraoptimal temperatures *E. coli* cultures accumulate extracellular protective metabolites that promote the adaptation of this bacterium to unfavorable conditions. It is known that *C. perfringens* adapts to high temperatures after a heat shock, however no information is available about the involvement of supernatant metabolites in this response. In this work we demonstrate the ability of *C. perfringens* to adapt to high temperatures by means of an extracellular factor that was released to the culture medium or food after heat shock. Cells were grown in a 4 ml fluid thioglycolate medium or chicken broth and incubated at 37°C. When cells reached mild-log phase, heat shock was applied at 50°C for 30 min. After that, cultures were centrifuged and supernatants were transferred to non-shocked cells. Heat tolerance of these cells was performed at 55°C. Viable cells were determined by plate count at different times. In some cases, 100 ml (1:250) of trypsin was added to the supernatants and incubated at 37°C for 30 min or the supernatants were heated at 65°C or 100°C for 15 min. The supernatant was fractioned using Nanosep 10 K and low pressure liquid chromatography was carried out. PAGE was made of fractions showing heat-protective activity. Results indicated that a heat shock applied to *C. perfringens* released compounds to the medium that conferred heat-tolerance to non-shocked cells in culture media and in chicken broth. The heat-protective activity was lost if the supernatant was treated with heat or trypsin. PAGE and Western blot analysis of the supernatant fraction with heat-protective activity indicated the presence of a 55kDa protein that cross reacted with GroEl antiserum. The mechanisms that confer adaptations of this bacterium to heat may be an important aspect of food safety because of the widespread application of thermal processing to the control of pathogenic microorganisms.

CHARACTERIZATION OF THE 461 AMINO ACID POSITION OF CUCUMBER MOSAIC VIRUS 1A PROTEIN RESPONSIBLE FOR NECROSIS INDUCTION

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The Ns strain of cucumber mosaic virus (CMV) induce necrotic lesion on numerous *Nicotiana spp*. in contrast to other CMV isolates that cause systemic mosaic on these plants. Earlier the cysteine at the 461st amino acid position of the 1a protein was identified to be responsible for this phenomenon. The three-dimensional structure of the 1a protein is still not known, only secondary structure predictions are available. This prediction shows a bended amphiphile helix between residues 460 to 472. Due to this fact we have further characterized the possible function of this helix based on modelling amino acid mutations. We have designed and introduced 7 different amino acid mutations into the 461st position of the infectious clone of RNA 1 of Ns-CMV. The infection characteristics of the different mutants were investigated in *N. clevelandii* protoplast and on *Nicotiana clevelandii, Nicotiana glutinosa* and *Nicotiana tabacum sc. Xanthi-nc* plants. The mutants C461E, C461P and C461N were not able to replicate in protoplasts. The mutants C461S and C461A caused similar lesions as the original Ns-CMV strain, while the C461R and the C461K mutations caused mosaic symptoms typical for most strains of CMV on all hosts tested. Among the non-infectious mutants, in the case of C461E, a negative charge appeared in this basic-neutral amphiphile helix in consequence of this mutation, disrupting the original charge of the helix. The proline in the mutant C461P breaks the helix, resulting in the non infectious nature of this mutant. The mosaic symptoms are associated with long, positively charged amino acids (C461R, C461K). The formation of a disulphide bond is not required for lesion development, since the C461S and C461A mutants induced lesions. We can conclude that the chemical property of the residue 461 of 1a protein plays a crucial role not only in replication but also in symptom formation.

ANALYSIS OF HISTONE H3 AND H4 ACETYLATION AND HISTONE H3-K4 METHYLATION AT THE LATENT EBV PROMOTER LMP2A

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Latency protein LMP2A of the Epstein-Barr virus (EBV) has been implicated in EBV related tumorigenesis. To understand the host cell dependent expression of the LMP2A gene, it is necessary to analyse the regulatory mechanisms of the LMP2A promoter (LMP2Ap). Because histone acetylation and histone H3-K4 methylation may lead to chromatin relaxation and subsequent modulation of gene expression, we examined the acetylation state of histones and the level of histone H3-K4 methylation at LMP2Ap on well characterized, EBV carrying type I, II and III cell lines with the method of chromatin immunoprecipitation (ChIP) assay combined with real-time PCR. These ChIP results showed that the active LMP2Ap contained more acetylated and H3-K4 methylated histones than the inactive ones.
Acknowledgement: This work was supported by grants T 042727 and F 048921 of the Hungarian Scientific Research Fund (OTKA). Dániel Salamon is a Bolyai fellow supported by the Hungarian Academy of Sciences.

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DETECTION OF AVIAN INFLUENZA VIRUS AND NEWCASTLE DISEASE VIRUS BY A NOVEL REAL-TIME RT-PCR USING LIGHT UPON EXTENSION (LUX) FLUOROGENIC PRIMERS

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Highly pathogenic strains of avian influenza virus (AIV) and Newcastle disease virus (NDV) cause contagious diseases in domestic poultry, including chickens and turkeys. AIV is member of the family Orthomyxoviridae; Newcastle disease virus belongs to the family Paramyxoviridae. In this study we report on two novel molecular diagnostic tools to detect AIV and NDV. Each of these Light Upon Extension (LUX), quantitative real-time PCR assays includes a single labelled forward primer with a JOE fluorophore at the 3' end in a hairpin structure, as well as a corresponding unlabelled reverse primer. LUX primers require no special probes or quenchers. The reliability of the assays is further supported by the melting-point analysis of the PCR products allowing the differentiation of amplicons and primer dimer artefacts by their melting temperatures. The specificity and sensitivity of the assays were tested on different viral strains, including vaccine viruses. Based on our results, the developed procedures can serve as sensitive and accurate diagnostic tools for rapid detection of AIV and NDV.
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MICROBIAL COMMUNITY ANALYSIS OF ACTIVATED SLUDGE TREATING INDUSTRIAL WASTEWATER

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Coke plant wastewater is produced in steel plants at coke-oven gas-cleaning operations. The produced wastewater contains high concentrations of phenols, cyanide and ammonia. These toxic compounds can cause severe pollutions in the environment. Therefore, the wastewater is subjected to chemical and biological treatment (activated sludge process), which usually removes phenols but the removal efficiency of thiocyanate, transformed from cyanide, is irregular. Ammonia concentration increases during the biological treatment . The aim of this study was to identify the members of the bacterial community and determine the microbial changes associated with the instable thiocyanate removal. First of all, we investigated the microbial homogeneity of the bacterial community of the six biological aeration basins by Terminal Restriction Fragment Length Polymorphism (T-RFLP) of 16S rDNA. The temporal changes in the bacterial community were also followed by T-RFLP analysis. A 16S rDNA clone library was created from the most characteristic sample and compared with the TRFLP pattern to identify the most numerous members of the bacterial community. Partial sequencing of the clones was used for phylogenetic identification.
Community fingerprint obtained by T-RFLP analysis shows that the aeration tanks were very similar with respect to microbial composition. Phylogenetic analysis of cloned 16S rDNA shows that the community was dominated by a strain closely related to a phenol degrading species, *Comamonas badia*. Members of the *Thiobacillus* genus are also present in the community and are probably responsible for the thiocyanate removal. The proportion of this group compared to other members of the community changes with the varying efficiency of thiocyanate removal. The produced ammonia is supposed to be connected to their activity.
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GENITAL HPV INFECTION: HEALTH IMPLICATION AND DIAGNOSIS

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Genital papillomaviruses (HPV) infections are among the most common sexually transmitted viral infections. Approximately 40 HPV types infect the anogenital tract and a few types are consistently found in anogenital cancer biopsy specimens, notably cervical cancer. HPV DNA has been identified in almost all-cervical cancer biopsies, among which HPV type 16 is found in more than 50% of cervical cancer cases worldwide. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are considered viruses with high oncogenic potential (high risk – HR), while HPV types 26, 53 and 66 are probably oncogenic. In contrast to HR HPV, HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108 are considered viruses with low oncogenic potential (low risk – LR).
The high prevalence of latent HPV infection among sexually active young women, and its significant decrease by 30 years of age reflects the transient nature of most cases of HPV infection. In contrast, persistent HPV infection over many years leads to genetic alterations, development of precancerous lesions and progression of those to cervical cancer. Thus, persistent HPV infection appears to play a central role in the pathogenesis of cervical cancer. As cervical pathogenesis develops slowly over many years, cervical cancer could be prevented and treated if detected in time. Therefore, HPV detection and typing represent useful and important tools for the diagnosis of HPV associated diseases and management of women at risk. The molecular methods used for HPV testing are based on the method of hybridization, DNA amplification or both. Several commercial methods, suitable for large scale HPV testing in clinical laboratories, are available. HPV testing detects prevalent infection and when

applied at age 30 or older, identifies those with a persistent type-specific infection. These women are at a high risk of either having or developing cervical precancerous lesions.
Recent clinical studies indicate that the combination of cervical cytology and HPV testing can significantly improve primary cervical cancer screening with a sensitivity of 100% for detecting cervical precancerous lesions. The very high negative predictive value of HPV testing allows longer screening intervals. Furthermore, HPV testing alone is significantly more sensitive than and as specific as conventional cervical cytology in primary cervical cancer screening. HPV testing alone may also be an attractive alternative to cytology in low resource countries with a high prevalence of cervical cancer.
Identification of cervical precancerous lesions through cervical cytology and/or HPV testing, and eventually their prevention through HPV vaccination and public health measures will lead to a significant decrease, if not complete eradication of cervical cancer in the next decades.

LONG-LASTING ANTIBIOTIC RESISTANCE IN THE NORMAL FLORA FOLLOWING CLINDAMYCINE TREATMENT

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To the question: "What is the target of an antimicrobial treatment?" any physician, or veterinarian would give the answer: "The patient" or "The pathogenic bacterial strain". However, the real effect we produce by treating a patient with antibiotics is much more complex. As our patient is an open ecology system, he is accompanied by his normal bacterial flora. This microbial community, when facing a selective agent, will answer via losing its sensitive components and proliferating the resistant ones. Thus, harboring a massively resistant normal (mainly skin and enteric) flora, the patient himself will be the main source of antibiotic-resistant strains. In the present work, we measured the proportions and time-course of such a resistant normal flora following Clindamycine (Dalacin C, 2x 300 mg/die) treatment. Using colony-count techniques and agar plate MIC method, the *Staphylococcus spp*. of patient's skin were examined. The 76%, 16%, 14%, 4% and 2% of CFU's were resistant against a high dose (16 ug/ml) of Clindamycine, on the 9th, 51st, 71st, 88th and 90th day, respectively, after finishing the therapy. Highly resistant *S. haemolyticus* and *S. epidermidis* strains were both present from the beginning of the experiment to the third month, suggesting that there was a real selection process, and not colonization with some specific hospital strain. The "antibiotic resistance phenomenon" is not a characteristic of any pathogenic strain, but rather a biological marker of environmental pollution, originating from the widespread use of antibiotic and disinfectant agents. After exerting their activity on the target site, 25-75% of these chemicals spread in the environment without degradation, making microbial communities uniform, destroying their functions in natural water and soil. We must recognize at last that bacteria are the base of Life on Earth. Thus, any antibiotic or disinfectant must be regarded as toxic, and handled as carefully as those with high human toxicity. We must recognize at last that hospital hygiene, handling needles and trays carefully, and releasing patients' feces into the common suburban sewer network, will not be successful in struggling against the emergence of highly resistant pathogens!
Acknowledgement: This work was supported by grant NKFP 1B/047/2004.

MORPHOLOGICAL, BIOCHEMICAL AND CULTURAL PROPERTIES OF HUNGARIAN *CLOSTRIDIUM BOTULINUM* STRAINS

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The earliest known form of human botulism is the foodborne disease caused by ingestion of an exotoxin produced by biovariants of *Clostridium botulinum*. These antigenically distinct exotoxin types **A**-**G** can block the release of the neurotransmitter acethyl-choline, thus nerve impulses cannot be transmitted. The result is a generalized flaccid paralysis and several vegetative symptoms. While serious cases need 14 to 21 days of hospitalization, mild forms are often stay unexplored. In Europe, thus also in Hungary, the majority of diseases is caused by inefficient processing of the meat of home-slaughtered pigs contaminated with *C. botulinum* type **B** spores.
In Hungary, foodborne botulism intoxications are noticeable since 1952. By now, 398 cases were reported, 5 of them lethal. First cases confirmed by demonstration of the toxin were reported by Nikodémusz et al. (1960) and Ralovich et al. (1966). Because of the numeral and geographical spread of foodborne botulism cases in our country, it would be useful for diagnostic and hygienic experts to know the cultural properties of Hungarian *C. botulinum* strains. Strains isolated by NIFSN are slow-growing and very sensitive to oxygen. Colonies on blood agar plate are flat with zygomorphic edges, and showing beta-haemolysis. On egg yolk agar, we can see polymorphic colonies of old cultures, and - beside the characteristic lipase reaction, lecithinase positive, lipase negative and lecithinase negative swarming colonies. In Holman broth, we can observe foaming and weak gas formation. In semi-solid peptone yeast broth, strains are weakly fermentative. Due to the matrix-requirement of these bacteria, they can only be cultured on agar- or gelatine-supplemented media. All strains isolated so far are sulphite-reducing. By virtue of biochemical and heat-resistance data, Hungarian *C. botulinum* strains recognized so far seem to be similar to Western European, biotype II. strains, which are **B** and **E** toxin producing, psychrophilic, nonproteolytic, saccharolytic, growing between 3-45°C, with a 25-30°C optimum. Typical biotype II. colonies, as well as the Hungarian ones examined so far, are beta-haemolysing, lipase positive, indole and lecithinase negative, fermenting glucose, mannose, fructose and arabinose characteristically, but not raffinose and salicine.

ENTRY INTO THE STATIONARY PHASE OF GROWTH INDUCES APOPTOSIS IN *CANDIDA ALBICANS*

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Development of apoptotic markers (membrane inversion and DNA fragmentation) was detected in stationary phase *Candida albicans* cultures. The elevated ratio of apoptotic cells was accompanied by increased intracellular superoxide and peroxide concentrations. Vitamin E did not cause significant changes in the growth of the yeast but it decreased markedly both the peroxide content of the cells and the ratio of the Annexin V positive, apoptotic protoplasts, suggesting that reactive oxygen forms were involved in the induction of stationary phase induced apoptosis.

Lovastatin is a well-known apoptosis inducer in mammalian cells and it also induces apoptosis in *Mucor racemosus*. In *C. albicans*, lovastatin had a dose dependent growth inhibitory effect and also caused morphological changes. In the presence of 12.5 mg/ml lovastatin, cultures showed pseudomycelial growth and hypha formation was also observed. However, lovastatin did not induce apoptosis in young cultures, and the ratio of Annexin V positive, apoptotic protoplasts was even smaller in old (24 h) lovastatin treated cultures than in the controls. It can be explained well with the growth inhibitory effect of lovastatin: In the presence of the drug, cultures did not reach the stationary phase of growth by 24 h and therefore they accumulated much less peroxides than the control cells.

EFFECT OF METHYLPREDNISOLONE ON THE PHYSIOLOGY OF *CANDIDA ALBICANS*

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Glucocorticoid-treated patients are susceptible to *Candida* infections since the defence mechanisms responsible for controlling fungal infections are dysfunctional after glucocorticoid therapy. Furthermore, it is also possible that glucocorticoids enhance the pathogenicity of *Candida albicans* directly by affecting the fungus's metabolism. The contribution of this effect to the increased pathogenicity after corticosteroid therapy represents a rarely studied area. In this work, we examined the effect of methylprednisolone (MP), a widely used corticosteroid in the therapy, on the *in vitro* growth and virulence attributes of *C. albicans* and its interaction with sterol biosynthesis inhibitors. We found the following: MP enhanced the growth, germination and extracellular phospholipase activity of the fungus, whilst it did not influence either the adhesive ability to plastic surface or the extracellular protease activity. MP did not modify the growth inhibitory effect of fluconazole but it counterbalanced the inhibitory potential of lovastatin. MP enhanced the growth in medium containing glucose but reduced it when the sole carbon source was glycerol. We detected the accumulation of MP into the plasma membrane of the yeast, which explains the changes in the utilization of glycerol. MP also increased the oxidative stress sensitivity of *C. albicans* cells dramatically. In the presence of MP, the growth inhibitory effect of menadione was much higher than that in control cultures. This property of MP can be explained well with our finding that MP elevated the level of lipid peroxidation products e.g. malondialdehyde and conjugated diene. In conclusion, our findings show that MP has a direct action on *C. albicans* metabolism *in vitro,* which may play role in the glucocorticoid-related enhanced pathogenicity of this fungus *in vivo*. A better knowledge on the interaction between glucocorticoids and fungal infections should assist in earlier recognition and treatment of *Candida* infections.

ANTIRETROVIRAL ACTIVITY OF ALKALOIDS ISOLATED FROM *LEUCOJUM VERNUM*

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Alkaloids - 2-*O*-acetyllycorine, 11-hydroxyvittatine, *N*-demethyl-galanthamine - isolated from *Leucojum vernum* species were tested *in vitro* for their HIV-1 replication inhibitory activity in the MT4 human T lymphocyte cell line. The cytotoxicity of alkaloids in uninfected cells (TC_{50}) was tested by

MTT cell proliferation assay. 2-*O*-acetyllycorine, *N*-demethyl-galanthamine, 11-hydroxyvittatine were toxic at TC_{50} concentrations of 6 µg/ml, 43 µg/ml and and 12 µg/ml respectively.
Following HIV-1 infection, the cells were grown in the presence of the alkaloids added in different concentrations, lower than the TC_{50} values. The inhibition of HIV-1 production was measured using a reverse transcriptase micro-assay. AZT was used as positive control in the experiments.
Decrease in virus production was seen with 2-O-acetyllycorine and 11-hydroxyvittatine The active compounds displayed 1.0 and 2.1 therapeutic indices (TI50), respectively. Cells tolerated *N*-demethyl-galanthamine in high concentration, but the alkaloid did not show antiviral activity. The reverse transcriptase enzyme was inhibited directly by 2-*O*-acetyl-lycorine, while *N*-demethyl-galanthamine did not inhibit enzyme activity

ENVIRONMENTAL MICROBIOLOGICAL STUDIES ON LANDFILL SITES

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Plant surfaces are colonized by a large number of various bacteria, yeasts and thread fungi. The bacterial count in the phyllosphere is frequently as large as 106–107 cell/cm^2. This study has aimed to examine the surface microflorae on the plants growing in contaminated areas, as well as to reveal the underlying factors influencing them.
In communal and industrial landfill sites, the quantitative and qualitative composition of contaminants is fairly diverse, and at the same time typical of the given landfill site, which can be regarded as a habitat providing an abundance of organic and inorganic materials to weeds and microorganisms living on their leaf surfaces. Sampling was performed at the landfill sites situated in the vicinities of various settlements in Szabolcs-Szatmár-Bereg County (North-Eastern Hungary). In the course of the studies, the local vegetation was assessed, and plants, such as *Urtica dioica*, *Arctium lappa*, *Galium aparine* and *Sonchus asper* were taken as subjects of the investigations. Having been adapted to the conditions of the given landfill site, leaf surfaces are highly peculiar habitats for microorganisms. The diversity of microorganisms is determined by various environmental factors, the microflora of the air and the soil, as well as the qualitative and quantitative composition of the wastes. Microorganisms living on the leaf surfaces of plants in these landfill sites have the potential to cause hazardous effects on human environments when scattered from the landfill sites by rain, wind and dusts.
Gram-negative bacteria isolated from the upper and lower leaf surfaces include those of the genera *Enterobacteriaceae*, *Vibrionaceae* and *Pseudomonadaceae*, while their Gram-positive counterparts are bacteria of the genera *Bacillus*, *Lactobacillus*, *Clostridium*, *Micrococcaceae*, *Mycobacteriaceae* and *Nocardiaceae*. Typical Gram-negative species found on plant surfaces are *Cryseomonas luteola, Pseudomonas cepacica, Pseudomonas flurorescens, Staphylococcus sciuri, Staphylococcus lugdunensis*. Gram-positive bacilli detected are *Bacillus cereus, Bacillus circulans, Bacillus subtilis*, *Bacillus megaterium, Bacillus licheniformis, Bacillus mycoides, Bacillus pumilus, Bacillus firmus*, while Gram-positive Coryneform bacteria include *Brevibacterum ssp*., *Oerskovia ssp*., *Corynebacterium aquation*, *Corynebacterium ssp*. The antibiotic sensitivity of bacilli isolated from the area of the landfill sites also highly varied. No significant amount of thread fungi and yeasts was found as compared to those of bacteria.

NOVEL BIOREACTOR SYSTEMS FOR MAMMALIAN AND PLANT CELL CULTIVATION

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Microbial fermentation has been used for centuries in the food-, pharmaceutical- and environmental industries. Conventional bioreactor systems are especially designed for bacteria, yeast and mold fermentation, and in some cases can be used for mammalian cell cultivation as well. Because of the sensitivity of most cell lines however, process parameters have to be chosen and controlled very carefully. Furthermore, the productivity of anchorage dependent cells is insufficient in such systems.
Cell culture fermentation is getting to become the center of interest as being a unique method in many areas of scientific research like genomics, proteomics, drug development of diagnostics, tissue engineering and environmental technology; and can be found in many different processes. Although cell cultures show slower growth rates and more sensitivity against environmental conditions, it is the primary method for the production of recombinant proteins. Because of their possibility for accurate protein folding, assembly and post-translational modification, the quality and efficiency of protein production is superior to other expression systems like that of bacteria and yeast. One of the main challenges nowadays is to develop automated reactor systems for anchorage dependent cell cultivation with a sufficient micro-carrier system assuring high productivity also in large-scale production systems. Sartorius BBI Systems Ltd. has developed a novel rotating bed fermentation unit – BIOSTAT® RBS - for the cultivation of anchorage dependent cells. A short overview and special system features are presented. Additionally, a brief introduction is given to recently developed photobioreactor system (BIOSTAT® PBR) designed for cultivating photosynthetic organisms like algae, moss or plant cells.

EXTRACELLULAR ENZYME PRODUCTION OF *TRICHODERMA* STRAINS CAUSING MUSHROOM GREEN MOLD IN HUNGARY

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Trichoderma species are common soil-inhabiting asexual filamentous fungi with teleomorphs belonging to the *Hypocreales* order of the Ascomycota division. Beside their industrial, agricultural and clinical importance, several isolates have been found to cause severe green mould disease in mushroom farms worldwide. Their effective competitive and mycoparasitic ability is highly related to the production of several extracellular enzymes. From 108 *Trichoderma* strains isolated from Hungarian *Agaricus* compost and *Pleurotus* substrate samples, the most aggressive 20 isolates were chosen for further studies based on their *in vitro* antagonistic abilities against *Agaricus bisporus* and *Pleurotus ostreatus*. The activity of 9 extracellular enzymes – β-glucosidase, esterase-lipase, β-1,3-glucanase, N-acetyl-glucosaminidase, chitobiosidase, endochitinase, as well as a chymotrypsin-like and 2 trypsin-like proteases – were determined and compared with 7 aggressive type strains derived from culture collection (CBS) after growing the strains in minimal medium, yeast extract-glucose medium and minimal medium supplemented with *Agaricus* or *Pleurotus* extract.

A great number of our isolates produced manifold levels of activities of certain extracellular enzymes when compared to the type strains.

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GENETIC DIVERSITY OF *TRICHODERMA* STRAINS AND OCCURRENCE OF *T. AGGRESSIVUM* IN HUNGARIAN MUSHROOM COMPOST AND SUBSTRATE SAMPLES

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Trichoderma species are imperfect filamentous fungi with teleomorphs belonging to the *Hypocreales* order of the Ascomycota division. They are well-known due to their industrial, agricultural and clinical importance. Furthermore, certain isolates have been found to cause green mold epidemics in mushroom farms. The most aggressive strains were originally identified as *T. harzianum* Th2 and Th4 in Ireland and North-America, respectively, but later they were redescribed as f. sp. *europaeum* and *aggressivum* of the newly introduced species *T. aggressivum.* Our aim was to study the genetic diversity of *Trichoderma* strains and the occurrence of *T. aggressivum* in Hungarian mushroom compost and substrate samples. A total number of 66 *Trichoderma* strains isolated from *Pleurotus* substrate (sample A) and *Agaricus* compost (samples B and C) deriving from 3 different geographic locations in Hungary were involved in the experiments. Genomic DNA was isolated from the full sample set, and a PCR-based test was performed with primers Th-F (5'-CGGTGACATCTGAAAAGTCGTG-3') and Th-R (5'-TGTCACCCGTTCGGATCATCCG-3') designed previously for the specific identification of *T. aggressivum*.

According to this test, 19 out of the 66 isolates proved to be *T. aggressivum*. Sequence analysis of the internal transcribed spacer region (ITS1-5.8S rRNS-ITS2) was carried out for all 66 strains in order to verify the identity of the putative *T. aggressivum* isolates and to study the genetic diversity of *Trichoderma* strains occurring in mushroom compost and substrate in Hungary. The identity of 16 out of 19 putative *T. aggressivum* isolates could be confirmed by ITS sequence analysis, demonstrating that the PCR-based test is appropriate for quick screening of *T. aggressivum*. However, some *T. harzianum* isolates also proved positive in the test, while a *T. aggressivum* isolate gave no fragment with the specific primers, suggesting that the identification of a strain as *T. aggressivum* based on this diagnostic primer pair has to be confirmed by further molecular techniques, e.g. ITS sequence analysis. Besides *T. aggressivum*, strains belonging to 5 further *Trichoderma* species could be isolated from the examined compost and substrate samples: *T. harzianum* (30 strains), *T. atroviride* (10 strains), *T. asperellum* (4 strains), *T. longibrachiatum* (4 strains) and *T. ghanense* (1 strain). Only *T. harzianum* could be isolated from sample C (*Agaricus* compost). Sample A (*Pleurotus* substrate) was also dominated by *T. harzianum*, but three further species (*T. asperellum, T. atroviride* and *T. longibrachiatum*) were also represented by single isolates. All of the species could be isolated from sample B (*Agaricus* compost) but *T. harzianum*, suggesting that the absence of *T. harzianum* may result in a greater diversity of *Trichoderma* species in mushroom compost.

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PSEUDOMONAS INOCULATION FOR IMPROVING THE FLOWER QUALITY OF GERBERA (*GERBERA JAMESONII*)

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Flower quality is the most important quality requirement of Gerbera production. Under greenhouse conditions, however, the deleterious effect of soil-borne plant-pathogens is known, against which the inoculation of the fluorescens-putida type *Pseudomonas* bacteria seems to be a progressive tool [1]. There was a question however to answer: which type of microbial inoculums could be efficient and how the inoculation could be developed in the most efficient way? Nine Gerbera varieties (*Kaukázus*, *Adamant*, *Uranus*, *Hanni*, *Extesie*, *Veronica*, *Amber*, *Rubijn*, *Atlas*) were tested in greenhouse using partially steam-sterilized- and non-sterile soil:perlite:peat 30:20:50 % substrates in a pot experiment. The five *Pseudomonas fluorescens* strains originating from the rhizosphere of the Gerbera were grown in other, healthy soil-plant systems. The isolates were selected for the pathogen control *in vitro.* Micropropagated 2-leaf-seedlings were put into 2 kg pots and the substrates, or the root systems were inoculated with the bacterial suspension with a level of 10^5 g-1 soil and/or root on a dry matter basis. The growth of plants was followed for 12 months after the microbial inoculation. The quality assessment of the flowers was performed twice a week.
Among the five preselected bacterium inoculums, two could produce an outstanding production of first-class flowers. This effect became significant in the non-sterilized substrates, at any inoculation practices. The other three pseudomonads, however, could produce better quality only in the sterilized substrates, which show that they are less competitive against the indigenous *Pseudomonas* population in the used soil. More efficient colonisation of the plant's rhizosphere was found in the presterilised substrate with all of the inoculums. Flower quality improved if the rhizosphere treatment was followed by substrate inoculation as well. The great variability of the results between the studied species and the inoculated strains or methodologies highlights the importance of strain-preselection and soil-monitoring when designing efficient inoculation technologies in the future.
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BIODEGRADATION OF KERATIN CONTAINING WASTES: A MOLECULAR APPROACH

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Huge amount of keratinous (animal feather, wool, hair) material is produced in Hungary annually. This waste is degraded very slowly in nature, therefore it is regarded as hazardous waste according to EU directives. Decomposition methods like incineration or chemical treatments are employed although these procedures are rather expensive or environment-polluting. In contrast, biotechnology offers environment-friendly and cheap degradation. Recently, our group isolated a keratin degrading

Bacillus strain, its extracellular keratinase enzyme was partially purified and characterized. Although a whole-cell based keratinaceous waste treatment method was developed with the isolated strain, the usage of pure keratinase might significantly reduce the degradation time and increase the yield of valuable amino acids in the ferment solution.
Here we show the isolation, characterization of the gene coding keratinase in *Bacillus licheniformis* KK1. Sequence analysis showed high homology with known keratinases and subtilisin Carlsberg. It is noteworthy that sequences of keratinases and subtilisin Carlsberg are extremely similar, in spite of the fact that the substrate specificity of the two enzymes differs significantly. This suggests that a small difference in the sequence, or another factor is responsible for the altered substrate specificities of these isoenzymes. Heterologous expression and mutagenesis of the keratinase would probably disclose the molecular reasons causing the different substrate specifities.
Therefore, several constructs were made in which the full-length gene was inserted into various overexpression vectors and introduced into *E. coli*. In these constructs, either the original *Bacillus* signal sequence or an alternative *E. coli* type signal sequence preceded the gene. For promoting the efficient purification of the recombinant enzyme, an affinity tag was fused to the C-terminal end of the protein. The optimalization of the overexpression systems is in progress.

INFLUENCE OF G.R.A.S. SUBSTANCES ON THE ACQUISITION OF COLD TOLERANCE IN *CLOSTRIDIUM PERFRINGENS*

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The use of chemical substances generally recognized as safe (GRAS) is a very common practice to enhance flavor or prolong shelf life of foods. Several factors occur during food processing that could modify the resistance of microorganism. It is known that bacteria exposed to non-favorable conditions induce synthesis of stress proteins that allow them to survive. Also, cross-protection has been observed between different treatments or stresses. In this work, we analyzed the effect of G.R.A.S. substances on induction of cold tolerance in *Clostridium perfringens* FD-1041 and FD-1. The G.R.A.S. substances of sodium benzoate, potassium sorbate, sodium nitrite, monosodic glutamate, or their mixtures were added (in amounts corresponding to the 25%, 50% and 75% of the maximum doses recommended for each G.R.A.S. substance) to cultures, then the effect on cold tolerance (cells were pre-shocked at 28ºC/60 minutes, and then treated at lethal temperature 10ºC) was evaluated. The results showed that sodium nitrite at 180 ppm stimulated the growth of *C. perfringens* and allowed it to tolerate the lethal temperature of 10ºC in the treated cultures. In the cultures treated with sodium benzoate 0.15%, *C. perfringens* acquired cold tolerance and no cold tolerance was observed when the recommended maximal doses of the other preservatives were used. After the assays with individual preservatives, we used mixes of them resulting in the following results: with a mixture of sodium benzoate 0.075% and potassium sorbate 0.1% added to the culture medium, we obtained cold tolerance, and the effect was similar when using the mixture of 0.375% sodium benzoate and 0.15 potassium sorbate, however in this case only strain *C. perfringens* FD1041 showed this effect; the mixture of sodium benzoate 0.075%, potassium sorbate 0.1% and 0.15% monosodic glutamate induced cold tolerance of both strains. The same results were observed when using sodium benzoate 0.1125%, 0.05% potassium sorbate and 0.15% monosodic glutamate. On the other hand, when 180ppm sodium nitrite was tested, only in combination with 0.20% potassium sorbate and 0.15% monosodic glutamate did the bacteria acquire cold tolerance.

THE ROLE OF TNF-ALPHA POLYMORPHISM IN *MYCOBACTERIUM BOVIS* BCG INDUCED TNF PRODUCTION

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TNF (tumor necrosis factor) plays an important role in the immunity against mycobacteria, and is considered to be pivotal for determining the course of the disease. Polymorphisms in the promoter of the TNF-a gene have been reported to affect the transcription rate and release of this cytokine.
The TNF-a inducing capacity of *Mycobacterium bovis BCG* in human white blood cells was determined, and the relationship between the *in vitro* TNF production and two polymorphisms of the TNF-a promoter regions was investigated.
Whole blood cultures of 50 blood donors were induced with *Mycobacterium bovis BCG* and *Staphylococcus aureus.* Thereafter the TNF-a in culture supernatants was determined by ELISA. The TNF-a -308 and -238 polymorphisms were determined by PCR and RFLP analysis.
There was a great individual difference in the rate of TNF-a production (350-10.000 pg/ml). The correlation between the -308 promoter polymorphism and a high level of TNF secretion was more striking than the correlation with the rare polymorphism at the -238 site. A whole blood culture is a convenient model for screening of cytokine producing capacity following different bacterial stimuli. TNF induction is not only dependent on the bacterial strain but is also determined by the host's genetic factors, which might influence the outcome of mycobacterial infections.

REPRODUCTION STRATEGIES IN *GIBBERELLA FUJIKUROI*

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Gibberella fujikuroi, sensu lato is a complex of eight mating populations (MPs), which are reproductively isolated from one another, therefore can be regarded as true biological species. Most MPs have recently been given distinct *Gibberella* species names and a number of asexual lineages, described as morphological species, have also been included into this complex group of fungi. These microorganisms are frequently associated with diseased plants and produce a variety of mycotoxins and plant growth promoting substances.
No sexual reproduction occurs between members of the different MPs or between the morphological species with no known sexual stage. Furthermore, sexual reproduction within the same MP can also be limited due to a lesser known phenomenon called female sterility. Like many other filamentous ascomycetes, strains belonging to the *G. fujikuroi* complex may also exchange genetic information through a parasexual manner. Heterokaryons are, however, formed only between clonally related strains, whereas unrelated lineages are vegetatively incompatible. Vegetative incompatibility is controlled by the *vic/het* genes, selectively neutral genetic determinants of somatic self/non-self recognition. Interestingly, vegetative self-incompatibility, preventing hyphal fusion and nuclear exchange, may also occur within the same strain, but neither the cause nor the mechanism of this type of incompatibility are known.
A series of cellular events is involved in both sexual and asexual compatibility, but the basic mechanisms, responsible for recognition, are still largely unknown. Recent investigations by the Gödöllő

Mycology Group allowed a better insight into these mechanisms: (i) a number of genes, regulated by the *MAT-2* (mating type) gene was tagged by differential DNA/DNA hybridization; (ii) a sensor protein, affecting both sexual and vegetative compatibility has been identified; (iii) the role of the cAMP-mediated signal transduction in sexual and parasexual events has been demonstrated and (iv) a het-c homologue involved in sexual but not in asexual compatibility has been characterized in details.
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USEFULNESS OF THE PCR METHODS IN THE DIAGNOSIS OF *PLASMODIUM* INFECTIONS

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The increase of tourism and population migrations has caused an increase of malaria cases reported in immigrants and travellers returning from areas where the disease is endemic. The detection limit of microscopy is only 5-20 parasites per ml and the mixed infections with more than one *Plasmodium* species are rarely identified by microscopic examination. Recently, the "National Reference Laboratory for Diseases Caused by Exotic Parasites" at the 'Johan Béla' National Center for Epidemiology has introduced two malaria polymerase chain reactions (PCR). One of them detects and identifies the malarial species with a sequence of two seminested-multiplex PCRs, and the sizes of the products are estimated after electrophoresis on agarose gel. The first reaction is expected to yield two products: the first being a band of 231 bp produced by the amplification of the human ribosomal gene, the positive control for each individual sample to prevent false negatives, and the second being a band of ca. 800 bp that should detect the presence of the ribosomal gene of any malaria species. Infections with different human pathogen *Plasmodium* (*P.*) species yield products of different sizes in the second PCR. In addition, we also introduced a real-time PCR assay. This method can detect the presence of the *Plasmodium* genus only, but the advantages of this method are sensitivity and fast turn-around time. Malaria PCRs have been carried out in 60 cases. The results of the PCRs were in harmony with the results of microscopic examination: The blood samples of 2 patients were infected with *P. falciparum* and 1−1 sample of 2 patients was infected with *P. vivax* and *P. ovale*, respectively and 1 sample of a patient was infected with *P. ovale* and *P. malariae*. Prevention of fatal outcomes in malaria cases requires early recognition of the infection, accurate laboratory diagnosis, and prompt therapy. The laboratory diagnosis of malaria by thin and thick blood smears as well as by real-time PCR is given in 3 h, so the physicians can apply the treatment as soon as possible and in the case of uncertain species identification, or in the case of co-infection, the seminested-multiplex PCR can help to distinguish the *Plasmodium* species.

IMPACT OF TOBRAMYCIN ON *ACINETOBACTER* STRAINS

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Over the past three decades *Acinetobacter* spp. have been more and more frequently responsible for hospital infections. Mainly *A. baumannii* may cause many serious infections. A high rate of antibiotic resistance was found in these strains and so the infections produced by these bacteria are difficult to

treat. Pharmacodynamic parameters – postantibiotic effect (PAE) and postantibiotic effect of subinhibitory concentrations (PA SME) represent important values leading to the optimal selection of drug dosing intervals. Antimicrobial agents inducing long PAEs and PA SMEs in infected organisms can be administered with longer dosing intervals than before without losing efficacy and with lower frequency of unfavourable reactions. Suppression of bacterial growth in seven strains of *Acinetobacter* species after 30 min treatment with tobramycin at suprainhibitory concentrations (PAE) and at supra-subinhibitory concentrations (PA SME) as well as changes in surface hydrophobicity and in the production of lipase and histamine in the exposed strains were studied. Pharmacodynamic parameters (PAE and PA SME) as well as modifications in tested bacterial characteristics were dependent on antibiotic concentration and on the strain used. Suppression of bacterial growth after treatment with tobramycin at 2x MIC was in the range of 0.6-4.5 h, a higher concentration (4x MIC) induced a longer PAE (1.9-5.4 h). Tobramycin at supra-subinhibitory concentrations (2x MIC +0.2x MIC and 4x MIC + 0.2x MIC) caused total inhibition of bacterial growth. In the majority of tobramycin –treated strains, an increase in hydrophobicity manifested by adherence of bacteria to xylene, as well as an increase in lipolytic activity was observed, but the production of histamine was not affected. Tobramycin at the tested concentrations suppressed bacterial growth that was associated with the change of some bacterial activities.

CLINICAL CHARACTERISTICS OF HUMAN ROTAVIRUS, ENTERIC ADENOVIRUS AND ASTROVIRUS INFECTIONS AMONG HOSPITALIZED CHILDREN IN BARANYA COUNTY, HUNGARY

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The aim of this study was to determine the prevalence, severity and clinical characteristics of viral gastroenteritis caused by astrovirus (HAstV), rotavirus (RV) or enteric adenovirus (EAd) resulting in hospitalization of children in Baranya County, Hungary. Stool specimens were collected between May 2003 and May 2004 from children hospitalized for gastroenteritis. Samples were tested for HAstV, RV, and EAd. HAstVs were detected by reverse transcriptase-polymerase chain reaction while RV and EAd were identified by latex agglutination test. Demographic and clinical data were collected for all enrolled children. Clinical symptoms and severity of illness were determined.
During this one year period, 227 children hospitalized for acute gastroenteritis were enrolled. The mean age was 37 months (range: 21 days to 213 months). HAstV, RV or EAd were detected in 52% of enrolled children. RV was detected in 94 (41%), EAd in 13 (6%) and HAstV in 12 (5%) stool samples. The most common clinical presentation of RV infected children were the combination of diarrhea, vomiting and fever (34%), while in children with HAstV or EAd infection diarrhea alone was more characteristic (50% and 62%, respectively). Most of the children infected with either virus had diarrhea lasting 1-7 days with 1-20 episodes per day, vomiting episodes lasted 1-5 days with 1-6 episodes per day. Children infected with RV had significantly higher fever ($\geq$ 38.0°C) than those children infected with EAd or HAstV. Based on the 20-point Vesikari severity score system; 58% of RV infected children had moderate or severe infection (score: $\geq$ 8) while 77% of children with EAd or 67% with HAstV had a rather mild disease (score: $\leq$ 7); ($p<0.05$).

Clinical presentations of gastroenteritis by RV, HAstV or EAd are similar with diarrhea being the most common manifestation followed by vomiting and/or fever. RV infected patients had a more severe illness than those children infected with EAd or HAstV. Based on the epidemiological and clinical data, we can conclude that these enteric viruses are a significant burden of disease based upon hospitalizations for childhood gastroenteritis in Baranya County, Hungary.

MORPHOLOGICAL AND MOLECULAR COMPARISON OF ECTOMYCORRHIZAE OF WHITE TRUFFLES (*TUBER* SPP.)

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White truffles are ectomycorrhizal ascomycetes, forming light-coloured, hypogeous fruitbodies. In contrast to black truffles of significant economical value (e.g. Tuber aestivum), white truffle species (Tuber borchii, T. maculatum, T. puberulum, T. dryophilum, T. oligospermum and T. rapaeodorum) are less studied concerning distribution, diversity and ecology. Although, white truffles are widely distributed in Europe, since the classical descriptions of Hollós from 1911, few new data based on sporocarps have been published about their diversity and abundance in the Carpathian Basin. However, our previous investigations of root samples collected in Hungarian forests showed that these fungi are common members of the ectomycorrhizal communities. Our work aimed at morphological-anatomical and molecular taxonomical characterization of ectomycorrhizae of white truffles collected from four different forest sites in Hungary (Tompa, Kelebia, Püspökladány and Bükk-Őserdő) involving the tree species Populus alba, Quercus robur, Q. cerris and Fagus sylvatica as hosts. Microscopic morphology was carried out by the methods of Agerer including stereomicroscopy, differential-interference-contrast microscopy (Nomarski-DIC) and phase-contrast microscopy (PhC), microscopical drawings and photodocumentation. Molecular characterization of the samples was carried out using sequence analysis of the nrDNA ITS-region. ITS sequences of 14 own mycorrhizal samples were analysed by appropriate phylogenetic methods (NJ, ML) together with GenBank-sequences obtained from fruitbodies. According to the molecular results, the investigated species segregated into two distinct clades corresponding species-groups. Nevertheless, the separation of the species within the clades is ambigous with respect to overlapping fruitbody characteristics. All ectomycorrhizae studied here were very similar in their micro-morphological features. Neither the ectomycorrhizae of the different species nor the two main groups could be distinguished.

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INACTIVATION OF FPMTR, AN UNUSUAL AMINO ACID TRANSPORTER GENE DISTURBS SEXUAL AND PARASEXUAL EVENTS IN *FUSARIUM PROLIFERATUM*

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In previous studies of this research group, a cDNA-AFLP approach was used to tag growth stage specific genes in *Fusarium proliferatum* (ITEM 2287), a worldwide distributed plant pathogenic, my-

cotoxin producing fungus. This investigation resulted in the identification of a putative amino acid transporter gene (*FpMtr*), strongly expressed during conidial germination and repressed in the late stationary phase. *FpMtr* showed significant sequence homology to *mtr* and *PcMtr,* neutral aromatic and aliphatic amino acid transporter genes known from *Neurospora crassa* and *Penicillium chrysogenum*, respectively. To find the specific function of this gene, *ΔFpMtr* knock-out mutants were generated by gene replacement.
Vegetative growth of the *ΔFpMtr* mutants was normal in liquid and solid media, but the germination of conidia was delayed, and abnormal germ tube development could be observed.
Male fertility of the *ΔFpMtr* mutants was not affected, however their female fertility became strongly retarded: the number of perithecia produced at days 21-28 of culturing on carrot agar decreased by 95%, as compared to the wild type. Strain ITEM 2287 is a vegetatively self-incompatible strain, i.e. complementary nitrate non-utilizing mutants (*nit1*, *nitM* and *nit3*) of this fungus are unable to form viable heterokaryons. However, when such mutants of a *ΔFpMtr* strain were paired with complementary auxotrophs of the wild type, normal cell fusion and heterokaryon formation were observed, indicating that inactivation of this interesting and unusual amino acid transporter gene abolished vegetative self-incompatibility. These data suggest that *FpMtr* is involved in multiple developmental processes related to both sexual and parasexual recombination events in *F. proliferatum* and FpMTR functions as a sensor/receptor protein rather than a typical amino acid transporter.
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TOOLS FOR THE ASSESSMENT OF FREE NITROGEN FIXER COMMUNITIES AND THEIR ACTIVITY IN THE RHIZOSPHERE OF GRAMINAE

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In continental biomes (climatic grasslands and agro-ecosystems), graminae and their root system sustain most of the free- living nitrogen fixers. Nitrogen fixation proceeds at the microbial scale through bacterial activity and may have an impact, on a larger scale, on the N cycle and plant biomass. Firstly, specific populations (*Azospirillum*) and communities of cultivable bacteria able to grow on synthetic medium without N supply were studied. Molecular tools were then developed and allowed the study of non cultivable free N-fixers (nifH gene). Genetic probes were designed to count populations (nifH probes) and to evaluate the potential of the rhizosphere bacterial communities to fix atmospheric nitrogen. Approaches to the expression of the function in a complex medium, like the rhizosphere environment, rely on the classical Acetylen Reduction Assay (ARA) and on mRNA quantification. Real-Time PCR was used to determine the ratio cRNA/DNA for a 360bp fragment of the NifH gene. Such tools ascertain the parameters of N fixation, including the number of bacteria being able to fix nitrogen or the efficiency of some populations, according to the rhizosphere conditions.

REDOX POTENTIAL MEASUREMENT AS A RAPID METHOD FOR HEAT DESTRUCTION EXPERIMENTS OF *CAMPYLOBACTER JEJUNI*

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The theoretical and practical basis of redox-potential measurement was used for heat destruction experiments. The assumption was that using both classical isotherm and anisotherm heat destruction models, the surviving (and injured) bacterial cells should be put in ideal circumstances, so we have to enrich them in broths used conventionally for the isolation of microorganisms. However, when using the enrichment technique – not plating directly onto agar surfaces – the problem of accurate determination of the original living cell number arises. The known disadvantages of the MPN technique could be solved using the redox-potential measurment method. The other advantage of this novel method over the classical techniques is the rapidness of the measurement.In our work, we studied the influence of the environmental factors on the thermal death of *Campylobacter jejuni*: the combined effect of pH, water activity and temperature was measured in anisotherm heat destruction models.
The anisotherm heat treatment model was carried out from 50°C to approximately 65°C, at three different pH and three different water activity levels. For the determination of the surviving cell concentrations, redox-potential measurement was used based on the previously determined calibration curves of *Campylobacter jejuni*. The D values were calculated from the survival curves (or from the TTDs). There were strict linear correlations between the logarithm of the D values and the temperatures, the z values were obtained from these equations. We can conclude that the redox-potential measurement method is a rapid, reliable and suitable tool – especially when combined with anisotherm heat treatment model – for carrying out heat destruction experiments.

SYNBIOTIC KEFIR BASED ON LACTOSE HYDROLYSED MILK, SUPPLEMENTED WITH JUICE OF *HELIANTHUS TUBEROSUM*

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Functional foods for specified health uses are produced for the maintenance of health in the digestive trackt and for the treatment of some metabolic diseases. Probiotics and prebiotics are considered to have positive health effects for many reasons. Probiotic bacteria, for example *Bifidobacteria* and many intestinal *Lactobacilli* are capable of fermenting prebiotics, resulting in metabolites such as lactic and acetic acid. These processes decrease the pH value in the large intestine and therefore reduce the number of harmful bacteria. Moreover, the useful probiotic species are able to produce bacteriocins against pathogen microbes. Prebiotics are defined as functional fibers, which can selectively stimulate the growth and/or metabolic activity of probiotic species in the gut. Synbiotics = specific probiotics and prebiotics. The type of prebiotic can determine the survival effects on the probiotics and their metabolites. The aim of our experiment was to produce a synbiotic kefir, fermented by probiotic bacteria and kefir yeasts, based on lactose-hydrolysed milk, supplemented with the juice of *Helianthus tuberosum*. This special kefir may be a functional food: the juice of Jerusalem artichoke (*Helianthus tuberosum*) is rich in fructose and inulin, so it is allowed for patients suffering from diabetes. These compounds and the mineral salts that occur in the juice of *Helianthus tuberosum* may be also useful for the probiotic starters. People, suffering from lactose intolerance are allowed to eat only special dairy products. Some fermented milks, based on lactose-hydrolysed milk, may give a good possibility to reduce not only lactose but also the galactose level to metabolise these carbohydrates by different kefir cultures. For this reasons, the lactose-hydrolysed milk samples were inoculated with

two types of mixed cultures: *Bifidobacterium breve* + *Lactobacillus rhamnosus* + *Kluyveromyces lactis* and *Streptococcus lactis* subspecies *cremoris* + *Lactobacillus paracasei* + *Kluyveromyces lactis*. (The lactose-hydrolysed milk was supplemented with 5% juice of *Helianthus tuberosum* before inoculation.) The fermentations were run at room temperature, until complete coagulation. The titritable acidity of the samples was measured during fermentation. The acetic acid content and lactose/galactose levels were controlled by Boehring-Mannheim enzyme tests after fermentation. The CFU of probiotic bacteria and kefir yeasts were obtained on China-blue lactose agar by pour plate method, during fermentation. The organoleptic characters of the samples were compared with traditional kefirs by the Kramer method. A higher acetic acid content and "Bifido" taste resulted in the samples, which were inoculated with *Bifidobacterium breve* in the mixed culture. The galactose level decreased below 450mg/100ml in all cases, so these special kefirs are also suitable for the diet of galactose sensitive people. The taste and aroma were significantly better in case of traditional kefirs than in case of synbiotic kefirs.

MICROBIAL PRODUCTION OF BIOFUELS FROM WASTES

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The only solution for reducing CO_2 emission joined with increased energy demand is the utilization of renewable fuels instead of fossil ones. A variety of alternative fuels can be made from biomass resources including the liquid fuels ethanol, methanol, biodiesel, and gaseous fuels such as hydrogen and methane. Since Hungary is poor in cheap, clean, high-quality domestic energy resources, only biomass can currently be considered as a significant source of renewable energy. Several lignocellulosic materials serve as possible feedstocks for the conversion to alternative fuel: by-products from the food- and paper industries, agriculture and forestry all provide various alternative energy sources. We aimed to test these wastes as possible feedstock for ethanol as biofuel, focusing on biological production methods. In these processes, enzymes and microorganisms are frequently used as “biocatalysts” to convert biomass into desirable products. Cellulase and hemicellulase enzymes break down the carbohydrate fractions of biomass to five and six-carbon sugars followed by fermentation of sugars into biofuel by yeast and bacteria. Pretreatment of lignocellulosic raw material before hydrolysis and fermentation is necessary to open up the structure, to make it accessible for enzymatic attack, thus increase the final yield. During this process a range of toxic compounds are formed: acetic acid is released during hemicellulose structure degradation, furfural, 5-hydroxymethyl furfural are produced due to degradation of sugars, while aromatic compounds originate from lignin degradation. Formic acid is formed when furfural and HMF are broken down and levulinic acid is formed by HMF degradation. All these toxic compounds (lignin and sugar degradation products) inhibit ethanol fermentation. In this study the fermentability of a *Saccharomyces cerevisiae* yeast strain was improved by adaptation to toxic components present in the pretreated lignocellulosic materials of spruce substrate. Results will be presented on the conference.

INVESTIGATION OF DIFFERENTLY TREATED SOIL TYPES BY NIR SPECTROSCOPY

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The application of sewage sludge is a common agricultural practice, because it improves water retention, reduces soil compaction and enhances biological activities on a "short-term" level.
Depending on its source, however, the sewage sludge often contains considerable amounts of heavy metals and organic toxicants that tend to accumulate with repeated „long term" application. The effect of sewage sludge deposition has been investigated from food safety aspects, and for the interaction between the beneficial microsymbionts (nitrogen fixers and phosphorous mobilizers) and greenpea (*Pisum sativum* L). The present study concentrates on NIR spectroscopy, as a fast and non-destructive method, which can be used for the characterisation and discrimination of soil. Four representative soils (calcareous sandy and chernozem; acidic sandy and forest) were treated with communal and industrial sewage sludge in doses of 0, 2.5, 5.0, 10 and 20 g.kg-1 soil ratios on an annual basis. In the fourth year NIR spectroscopy analysis was used by the PQS and SRT methods, evaluated by principal component and canonical discriminant analysis (PCA, CDA), respectively.
There were well-characterised and distinct properties found in general, regarding the representative soil types of Hungary. The main soil classification results can have the most numerous effects not only on the limits of sewage sludge depositions, but also on the survival of microbes of food-safety importance. Such measured parameters of the soils seemed to be independent of the applied sewage sludge doses, although the sandy and loamy type of soils behaved somehow uniformly in this respect. In addition to the soil types, all doses of both sludge-types could be perfectly identified with NIR analysis in comparison with the physical and chemical changes of the soil. The microbiological properties were found to be the most variable parameters, as a function of the different microbes, their rhizosphere location and their main eco-physiological characteristics. Due to the general sensitivity of microbes of food-safety importance, no significant accumulation was detected. However, the analysis used could be discriminative not only for the soils, but also for the different sludge-types and the different appliction doses, which may help to define the permissible limits of sludge depositions in main types of Hungarian soils.
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CELLULAR DISTRIBUTION OF ACCUMULATED HEAVY METALS IN DIFFERENT YEAST SPECIES

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Different yeast species are able to accumulate heavy metals in their cells. In the case of living cells, both extracellular and intarcellular accumulation takes place via the process called biosorption. Our aim was to determine the ratio of the surface accumulated and intracellularly enriched heavy metals in the case of *Kluyveromyces lactis*, *Schizosaccharomyces pombe, Lipomyces kononenkoe, Geotrichum candidum* and *Rhodotorula mucilaginosa.* DEAE-dextran as a permeabilizing agent was applied and the successive permeabilization of the cytoplasmic and vacuolar membranes was performed by the modified version of White's and Gadd's method.
The extracted microelement fractions were as follows:
- *Cell wall bound (chelated) fraction*: cell extract obtained after EDTA-Na2 treatment

- *Cytosolic fraction*: cell extract obtained after treatment with sorbitol-DEAE-dextran
- *Soluble vacuolar fraction:* cell extract obtained after 60% methanol treatment
- *Organically bound fraction:* cell sediments obtained after 60% methanol treatment

According to our results, a relatively low ratio of heavy metals, adsorbed by the cell wall as cations and removed by EDTA as a chelating agent, was obtained. *Geotrichum candidum* contained in this fraction the highest ratio of metals (between 11 and 23 % of the total accumulated metal). Cytoplasmically dissolved fractions also represented a very low ratio. There were some exceptions but we could not exclude that these higher values were errors of the applied method. This method has been elaborated and used for *S. cerevisae* but the osmotic susceptibility of different species could be different. 0.7 mol sorbitol and DEAE-dextran treatment can result in the disruption of intracellular membrane before the next osmotic shock. Vacuolar fractions of the accumulated metals were also low in ratio, except for lead. Organically bound fraction represented the highest ratio (between 43 and 94%), which consisted of the cell wall bound and the intracellularly bound subfractions.

SPECIFIC GROWTH RATE – AN ELUSIVE BUT CRUCIAL FACTOR IN THE REGULATION OF FUNGAL METABOLISM

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The parameter that represents the rate of growth per unit amount of biomass is termed the specific growth rate and has the dimension of reciprocal time. While the quantitative determination of transient growth rate in a batch culture is difficult, some hints towards its effect on the metabolism of filamentous fungi may be deduced from the fact that several industrial fermentations are traditionally performed in two phases. First, a rapidly metabolised carbon source favours biomass formation, to be followed by the supplementation of carbon sources allowing only slow growth but a high rate of product formation. It was therefore proposed, that the specific growth rate per se might influence fungal metabolism. We have tested this hypothesis for two cases: one was carbon catabolite repression of the formation of beta-galactosidase activity by D-glucose. In glucose-limited chemostat cultures of *Aspergillus nidulans* and *Trichoderma reesei*, beta-galactosidase formation is repressed only at high and moderate specific growth rates (D = 0.075 h-1, 0.050 h-1 and 0.030 h-1), but derepressed at low growth rates (D = 0.015 h-1). Chemostat cultures with a carbon catabolite derepressed *A. nidulans* mutant strain, on the other hand, revealed a dilution-rate independent constant beta-galactosidase activity of the same range as that found in the wild-type strain at D = 0.015 h-1. Thus the creA (*A. nidulans*) or cre1 (*T. reesei*)-dependent carbon catabolite regulation is a growth rate dependent process. The second example is lactose (1,4-O-β-D-galactopyranosyl-D-glucose) induction of cellulase formation in *T. reesei*. Although lactose must be hydrolyzed before it can be taken up by the fungus, recent data showed that neither D-glucose nor D-galactose induces cellulases in batch cultures. To test whether either of these sugars may be an inducer only at low growth rates (which are typical for cultivations on lactose), chemostat cultivations were performed at different dilution rates on D-galactose, lactose as well as D-glucose, and cellulase gene expression was monitored in a cbh2:goxA reporter strain. In addition, the extracellular accumulation of the two major cellobiohydrolases, Cel7A and Cel6A, was investigated by Western Blotting. The results show that while D-galactose induces cbh2 gene transcription and Cel7A/Cel6A accumulation at D = 0.015 h-1, lactose does so more efficiently and even at higher growth rates (D = 0.042 h-1). Traces of Cel7A, encoded by the partially Cre1-regulated cbh1, were detectable at D = 0.015-1, which led us to conclude that the triggering of cbh2 gene expression by D-galactose at 0.015 h-1 is not due to carbon catabolite derepression, but

true induction. Our results also imply that the specific growth rate of cultures is a crucial parameter that might have an impact on a wide array of regulatory mechanisms.

GENETICALLY RELATED CLUSTERS AMONG HUNGARIAN *ESCHERICHIA COLI* O157 EHEC AND EPEC STRAINS

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Enteropathogenic (EPEC) and enterohaemorrhagic *Escherichia coli* (EHEC) are the major causes of infectious enteritis throughout the world. The EHEC infection is zoonosis, with cattle being the major source of infection. Both meat- and milk-derived outbreaks were reported. Possible alternative sources of infection are small ruminants and rabbits. As a significant proportion of EPEC and the majority of EHEC strains belong to the serogroup O157, we concentrated our work on *E. coli* O157 in order to determine the possible genetic relatedness of Hungarian isolates.
We examined 46 Hungarian bovine isolates (31 EPEC, eight EHEC, one Shiga-toxin producing (STEC) and six toxin and intimin negative strains). The six toxin and intimin negative strains and five EPEC strains originated from a dairy herd and were isolated from feces and raw milk sample pairs of randomly selected animals. The other 35 isolates originated from a number of different cattle stocks and were isolated from fecal and colonic samples of the same animals collected at different slaughterhouses. We also examined four rabbit and one porcine EPEC isolates. Furthermore, we included five porcine enterotoxinogenic O157 strains isolated in Hungary or Austria as well as five human EHEC strains isolated in different countries as epidemiologically independent controls.
In order to study the genetic relatedness or diversity of these strains, PFGE based XbaI macrorestriction analysis and subsequent fingerprint analysis was performed. For clustering of the isolates, we used the Dice similarity coefficient and the UPGMA method.
Among bovine isolates, we could demonstrate three distinct clusters, one consisting of 20 EPEC isolates, another including six EHEC isolates and the STEC isolate, while the third cluster comprised of four milk-derived isolates from the dairy herd. Beside this cluster, the herd harbored other six different strains of *E. coli* O157, and all of them differed from isolates of other herds. Interestingly, only one milk-derived isolate proved to be genetically related to a fecal isolate, which originated from another animal. The non-bovine isolates and the other 19 bovine strains were found to be independent of the clusters, and - except for several related pairs - they were unrelated. These results show that a significant proportion of Hungarian *E. coli* O157 isolates is clonally related, similarly to results from other countries.

GENETIC DIVERSITY OF *CANDIDA ALBICANS* STRAINS ISOLATED FROM ORAL, URINE AND BLOOD SAMPLES

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We investigated the genetic relatedness of 71 *Candida albicans* strains isolated from oral, urine and blood samples in the University of Debrecen. Twenty-four oral and 20 urine isolates from different patients were randomly selected out of 189 and 62 strains, respectively, isolated between January and June, 2005. Blood isolates were collected through 1998-2005, and all 23 isolates from different patients were included in the study. For assessment of relatedness we used randomly amplified polymorphic DNA and computerized analysis of banding patterns using the Dice coefficient and the UPGMA method. We included two strains in all PCR reactions and used them as references in all analyses. Parameters were set so that reference patterns would show at least 96% similarity.
Using these settings, we found six clusters of possibly related isolates. Regarding the origin of the samples, these clusters were heterogeneous, indicating that presence of clones adapted to certain body sites is unlikely. Frequent relatedness among urine isolates, however, suggests frequent nosocomial transmission of candiduria.

MICROBIOLOGICAL CONSEQUENCES OF A MONO- OR TRICULTURE CROP-ROTATION EXPERIMENT

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The effect of monoculture and crop-rotations, such as maize monoculture (*Zea mays* L) and the triculture of soybean (*Glycine max* L) - maize - wheat (*Triticum aestivum* L) series, was studied on a calcareous chernozem soil in Látókép, Debrecen, Hungary, regarding the consequences in the physical, chemical and biological properties of the soil. Beside soil composition and compaction, soil acidity, humus content and nutrient availability were measured regularly for 18 years in a long-term field experiment. The plots in the experiment received different fertilizer doses [control (0), small (N60P45K45), small - medium (N120P90K90), medium-large (N180K135P135) and large (N240P180K180)] annually, before vegetation periods each year. The soil- (rhizosphere) microbiological properties, such as the total heterotrophs and micromycetes, the cellulose-decomposers and nitrifiers were assessed at 17th and 18th years by classical methods, described by Szegi [1]. Total- and specific soil-biological community analyses were done by microbial biomass, carbon-dioxide release and enzymatic measurements, such as phosphatase, saccharase, catalase and urease activity. More details of the experiments in [2].
Soil pH and acidity were decreasing in the fertilized plots. Such reduction was found to be more pronounced in the mono-, than in the tri-culture crop rotations. The same results were found in case of the nutrient contents (available N, Al-soluble P-, and K-content) of the soil, concomitant with a dose-dependency at the medium- or higher fertilizer applications. Countable or MPN (most probable number) density of microbes (heterotrophs, nitrifiers and cellulose-decomposers) was incresing in parallel with the improved nutrient availability in fertilized soils. A higher microbial density was found in the tri-culture treatments than in monoculture, while the microbial biomass measurements and some of the enzyme (i.e. saccharase) activities did not show differences.
There was also an opposite tendency recorded among the increasing phosphatase, urease and decreasing catalase activities in the fertilized plots. In this respect, crop rotation as triculture reesulted in a higher microbial activity in all treatments in comparison with the maize monoculture. The differences in the enzyme activities can be related to their eco-physiological specificities. The importance of the host-plants in the same soil-fertilizer treatments is being further highlighted in the study.

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DO HUMAN CALICIVIRUSES CAUSE EPIDEMICS EXCLUSIVELY?

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Among the small round structured viruses (SRSVs), such as the rota-, adeno- and astroviruses, the human caliciviruses (HuCVs) are the most frequent aetiological agents of acute non-bacterial gastroenteritis worldwide. The sources of infection are stool contaminated water, food and aerosol. A very rapid spread can occur within closed communities, because 10-100 virus particles are sufficient for infection. The HuCVs can be divided into two genera: Norwalk- and Sapporo- like viruses. With traditional methods, the HuCVs cannot be cultivated.

The aim of this study was to compare PCR and real-time PCR (SybrGreen), and two different primer pairs. The primer pair p289 and p290/A, which recognize RNA-dependent RNA polymerase, of the conserved region of the virus was used for PCR methods (Jiang). This primer pair was also adapted for real-time PCR (SybrGreen). Following the development and application of a mathematical algorithm, a new primer pair was introduced for real-time PCR (SybrGreen), and its use was compared with the former methods.

Amplified products were subject to sequence and phylogenetic analysis.

Between January 1, 2003 and June 30, 2005, 1357 stool samples were analysed. The standard laboratory procedure for SRSVs was used. All stool samples were examined for rotaviruses, all negative samples for adenoviruses, all negative samples for astroviruses and all negative samples for HuCVs. The prevalence of the rota-, adeno-, and astroviruses and HuCVs were 6.04%, 3.68%, 1.33% and 13.45% respectively. Sequence and phylogenetic analysis revealed that the 58 HuCV isolates included some very rare strains.

110 clinical samples were compared via the primer pair p289 and p290/A and the newly introduced primer pair. 6 HuCV strains (5.4%) were detected with the Jiang primer pair, and 37 (33.6%) with the new pair. By using the new primer pair, we detected a higher number of positive samples, and a lower prevalence of non-specific products.

OCCURRENCE OF SHEA (CLYA) GENE IN *ESCHERICHIA COLI* STRAINS

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The pathogenic role of silent or cryptic hemolysin of *Escherichia coli* has recently attracted increasing attention. The toxin forms a pore on erythrocyte membranes and induces apoptosis in macrophages. It is encoded by the sheA (clyA or hlyE) gene located on the chromosome. We investigated

the occurrence of this gene in *Escherichia coli* strains isolated from human (830) and animal (36) samples and of the ECOR collection (72 strains) by PCR technique. The samples of human origin were collected from intestinal (180) and extraintestinal (540) infections, and from healthy subjects (110). The results were compared to that of Ludwig studying 81 strains. According to our results, 94 % of the pathogenic and 85 % of the apathogenic intestinal isolates carried the sheA gene, while less than half (44.6 %) of the strains recovered from extraintestinal infections gave a positive PCR signal with the sheA-specific primers. In contrast to the results of Ludwig, we found that in some EIEC and ETEC strains IS elements were inserted into the gene and also suffered deletions. All of the strains (14) originating from oedema disease of swine carried the sheA gene, while only 50 % of the strains isolated from urinary tract infection of dog (22) were positive.

ANALYSIS OF FUNGAL POPULATIONS OF A BIOFILTER TREATING REFINERY WASTE GAS

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In the last few years, liquid and gaseous wastes of MOL refineries are treated increasingly with biological processes. Performance of the biofilters mainly depends on the adaptation of the degrading microorganisms to different organic pollutants (aromatics, paraffines, oxygenates, halogenated and sulphur compounds) and to the physical and chemical conditions. The filter beds are recently being inoculated with cultures with high degrading capacities, involving several fungal species in charge of decomposition of volatile organic compounds (VOC) in environmental technologies. Taxonomic and ecological analyses of the mycota revealed their role in the biofilter system for eliminating VOC in the oil industry.

Samples from the filter media/material of the filter bed were investigated by microbiological culturing, while the concentrations of volatile compounds in inflow and outflow gases were followed by gas-chromatography. Four weeks after the intensification, the efficacy of VOC removal significantly improved (from 52.6% to 80.3%). The amount of saprofitic bacteria, indicating the level of microbial biomass, also grew (from 3.9×10^5 CFU/g to 1.3×10^7 CFU/g). The suprisingly high representation of mycota (7.9×10^5 CFU/g) in the filter media of the active biofilter points to its outstanding role. Four mesophilic strains belonging to three species (*Cladosporium sp*., *Paecilomyces variotii*, *Paecilomyces sp*.) and three thermophilic strains of three different species (*Malbranchea cinnamomea*, *Thermoascus aurantiacus*, and that of a dark, septate nonsporulating type) proved to be dominant. Their frequent occurrence in the filter bed suggested that these fungal strains tolerated not only the high concentrations of volatile pollutants but also they could successfully metabolize them.

TAGGING TARGET GENES UP-REGULATED BY THE MAT-2 PRODUCT IN *FUSARIUM VERTICILLIOIDES*

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In filamentous ascomycetes, mating type is under the control of a single mating type locus (*MAT*) with two functional idiomorphs, named *MAT-1* and *MAT-2*. Transcription factors, encoded by the *MAT* genes regulate a number of other genes involved in cellular communication and morphogenetic changes needed for mating. *Fusarium* species with no known sexual stage also have structurally intact, functional mating type genes, therefore the MAT products may also regulate other types of genes not involved directly in the mating process. In order to identify putative target genes of the *MAT*-derived transcription factors, *MAT-2* knock out mutants were produced by transformation using a *hph* (*hygromycin phosphotransferase*) cassette. To analyze the differences in transcript profiles of a mutant and the wild type, the two strains were grown on carrot agar media known to stimulate mating. mRNA was isolated from five-day-old mycelia and cDNA libraries were prepared from both strains. Random clones representing the total genome were prepared from genomic DNAs of the two strains, transferred to high density filters and hybridized to radioactive probes prepared from cDNAs of the mutant and the wild type. Under such conditions, differences in signal intensities indicate transcription differences between the two strains. Altogether, 171 clones gave significantly higher signal intensities in the wild type as compared to the *ΔMAT-2* mutant, i.e. these clones probably are fragments of genes, stimulated by the MAT-2 product. The nucleotide sequences of these clones were compared to database sequences using the NCBI Blast Service. According to sequence similarities, 21% of the clones gave matches to proteins involved in amino acid/protein metabolism, another 21% could be involved in cellular communication and signaling, 15% in carbohydrate metabolism, 12% in stress-response, 6-6% in transport, energy, and RNA synthesis, and 3% in cellular organization. Ten per cent of the clones showed homologies to proteins of miscellaneous functions. Several clones were redundant: a nitrilase homologue occurred 20 times, an a-glucosidase appeared in triplicate, whereas duplicated copies of a nitroalkane oxidase and a phosphoenol-pyruvate synthase homologue were also identified. Sequences appearing in redundant copies and/or showing strong expression in the wild type are the most promising clones that are probably derived from genes involved in pheromone/receptor biosynthesis and photoinduced regulation of cellular events.

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FOOD SAFETY AND MICROBIOLOGY
(SPECIALIST POST-GRADUATE COURSE AT CORVINUS UNIVERSITY OF BUDAPEST)

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Food safety is today a basic requirement in the fields of production and sale of food. Thus high professional knowledge is needed from the producer of raw materials, to the manufacturer, catering trade, commerce and control. Food is safe, if it is not dangerous to the health of the consumer and does not cause illness. These requirements can not be fulfilled without the knowledge of the latest results in biology, chemistry, physics, technical sciences and their interactions. The solution of the problem is of an interdisciplinary character. People working in this field need a systematic and special post graduate education over the basic knowledge that the current requirements are able to fulfil.
In this form a first of its kind – in the country and looks like in Europe as well – a specialist post-graduate course for food safety started in the Faculty of Food Science of Corvinus University of Budapest in September 2000. The aim of the course was not only to change the attitude to food produc-

tion but also to give knowledge and apply it in a creative way in practice. Those who have a BSc or MSc degree in the field of food and agriculture, health, chemical or biological areas, and have some practice, can participate in the course. In our specialist post-graduate course, all of those new biological, chemical and physical factors (such as hazardous components) are dealt with, which may be present in raw materials and in processed or ready-to-eat foods, or may be formed during processing and storage. At the same time, requirements and control systems are are also dealt which are able to minimize these hazards. The involvement of the knowledge on raw materials, establishing area of processing plants and their control, as well as that of food safety, hazard analysis, risk assessment, risk management and HACCP, including their relations with legislation and regulations, international aspects, and related management tasks. The training period lasts three semesters and during the forth semester the trainees write a specialized thesis. The participants obtain their specialist diploma based on their oral examinations at the end of each semester, a final examination and the open discussion of their specialized theses.

This course devotes special importance to food microbiology. A higher percentage of food-borne illnesses are of microbiological origin. During the course, the relationship between microbiological safety, food production and sale are made very clear. Safety of final products can be obtained only from high quality raw materials, with good microbiological quality, good and effective technologies and adequate hygienic conditions. In order to obtain safety of food it is very important to know spoilage and illness causing microorganisms, viruses, parasites, which may be present in food, and properties of their metabolites, the efficiency of the different treatments, preservations, the effect of ecological factors and the effect of disinfection.

VORICONAZOLE, THE NEW PROMISING AGENT FOR ANTIFUNGAL THERAPY - EXAMINATION OF IN VITRO SUSCEPTIBILITY TO VORICONAZOLE OF FUNGI

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Fifty years ago, Dr. Anna Csillag established the department of Mycology to help physicians diagnose opportunistic fungal infections and to choose the adequate therapy. Since then, the number of fungal infections have increased, the methods have been improved and the spectrum of antifungal agents has become wider. One of the new agents for antifungal therapy is Voriconazole. Last year the examination of in vitro susceptibility to Voriconazole of fungi started in our laboratory. First 45 yeasts were examined (11 *Candida albicans*, 10 *Candida glabrata*, 8 *Candida krusei*, 2-2 *Candida lusitaniae* and *Candida parapsilosis*, 1-1 *Candida zeylanoides* and *Pichia farinosa*) with E-test on Casitone-agar medium. 95.6% of yeasts (43) had MIC equal or lower than 1 µg/ml, and only two of them (4.4%) had a higher MIC than 1 µg/ml. Furthermore, microcolonies within a discernible ellipse could be observed at all of *Candida glabrata* strains, and some of *C. krusei* and *C. tropicalis* strains. If these cases are regarded as MIC>32 µg/ml, than 26.7% of the examined yeasts had MIC > 1 µg/ml. As interpretive breakpoints have not yet been established for voriconazole, we have elected to use the following criteria after our examinations: Sensitive: MIC 1 µg/ml. After these series of examinations, the susceptibility tests to voriconazole of yeasts and moulds isolated from clinical samples started. 44 tests were made (22 moulds, 22 yeasts) from April 2004 to July 2005. In 38 cases (86,4%), the fungus was sensitive to voriconazole. Only 4 moulds (2 *Aspergillus fumigatus*, 2 *Fusarium sp.*) and 2 yeast strains (1-1 *Candida krusei*, *Candida parapsilosis*) had higher MIC than 1 µg/ml.

CHLAMYDIA PNEUMONIAE GENE EXPRESSION IN HUMAN DENDRITIC CELLS

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A common feature of all chlamydial infections is that they are often asymptomatic and may persist for long periods of life if left untreated. *Chlamydia pneumoniae* (Cpn) causes community-acquired pneumonia and is associated with such severe chronic diseases as atherosclerosis and asthma. Dendritic cells (DCs) are the most important members of the professional antigen presenting cells and play a crucial role in initiating primary immune responses. It has been shown that DCs are predominantly present at the sites of predilection of atherosclerosis plaques in the intima of the arterial vessel walls in young children. In vitro cultures of human macrophages support a limited growth and persistence of Cpn.
To our best knowledge, no experimental results have been published on the interaction between human DCs and Cpn infection. We wanted to find out whether Cpn can persist in DCs. Chlamydial organisms undergoing productive infection have a different gene expression pattern than during persistency. We used real-time RT-PCR approach to study the differential level of chlamydial gene transcription for six genes, such as 16S RNA (house keeping gene), GroEl (coding cHSP60 homologous protein, expressed in early stage), ompA (coding major outer membrane protein, expressed in middle stage), omcB (coding cysteine rich protein, expressed in late stage), and ftsK and ftsW (cell division-related) genes. Monocyte populations were isolated from peripheral blood mononuclear cells by the adherence method and treated with GM-CSF and IL-4 for 7 days for DC maturation. At day 7, DCs were infected with Cpn at a multiplicity of infection of 3 and were harvested at several times post infection. The control cell line HEp-2 was infected under the same conditions. RNA was isolated and treated with DNase before reverse transcription. We applied nested primers obtained from literature and other nested primers that were planned by Dnastar. Each primer set was tested to confirm that chlamydial but not host cell sequences were amplified. A standard curve was generated for each gene and the bacterial mRNA expression level was normalized to 16S RNA. The outer nested primers were used to create amplicons to measure the copy number by spectrophotometer. The mRNA expression of chlamydial genes in DCs and HEp2 cells was quantitatively determined and compared. The results are discussed.
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MORPHOLOGICAL TRANSFORMATION OF *CAMPYLOBACTER JEJUNI* UNDER STRESS CONDITIONS

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Campylobacter jejuni, a microaerophilic, gram-negative and thermotolerant bacterium is a common cause of gastrointestinal disease in humans. Still unknown regulatory networks allow the cells to dynamically adapt and survive adverse environmental stresses. *C. jejuni* undergoes a dramatic morphological transformation from a spiral to a coccoid form in response to unfavourable conditions. This

could have an important influence on survival, dissemination and virulence properties, as well as on reliability of detection of *Campylobacter* cells.
Some questions regarding the metabolic activity of coccoid cells remain open. For microbiological safety assessment during the food production chain it is therefore important to include alternative detection methods based on criteria other than reproduction. Phase contrast and fluorescent microscopic studies were used to study connections between viability and morphological transformation under stress conditions (starvation, heat shock, oxygen and peroxide stress).
We could reveal that different physiological and morphological forms predominated after exposure to different stresses. Through transmission electron microscopic (TEM) observation after negative staining, morphological transformations of the cells were confirmed. Campylobacters lost spiral shape and short cells with lack of cell integrity were found under exposure to stress. Our data support the theory of our previous studies that heat shock and especially starvation and oxidative stress provoke transformation to different coccoid cells, while some cells remain viable. The influence of stressed damaged cells on detection error using different microbiological and molecular biological methods as well as virulence properties of stressed cells remains objects of further studies.

EPSTEIN-BARR VIRUS -B LYMPHOCYTE INTERACTIONS

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EBV was discovered in a B cell malignancy-Burkitt lymphoma (BL) in 1965. Later it was identified as the causative agent of infectious mononucleosis (IM). After the discovery of its B cell transforming and immortalising capacity in vitro, attention focused on the analysis of its growth promoting activity. The function of the viral genes and their collaboration with each other and with cellular genes, were the first targets of these studies. While in transforming B cells the virus is highly efficient in vitro, it rarely induces B cell proliferation in vivo, mainly due to immunological control mechanisms. The immune response against the virally induced proteins and the phenotype of the transformed cells that promote interaction with the cells of the immune system permits manifest lymphoproliferation when the immune system is impaired.
Several haemopoetic, epithelial and mesenchymal tumor types were found to carry the virus. However, the role of the virus in the majority of virus positive malignancies remains unknown. Six nuclear (EBNA) and three cell membrane localised (LMPs) proteins were characterised in the LCL. This expression pattern is called Type III or growth program. All nuclear proteins, except for one, are essential for the transformation of B lymphocytes. EBNA-2, being the most important, together with LMP-1 alters the cell phenotype and induces proliferation. In addition to the Type III viral program, B cells with three further combinations of viral protein expression can be detected in lymphoid tissues of IM patients. Results obtained with tumor derived lines show that only the Type III viral strategy induces cell proliferation without the contribution of additional factors. BL cells express the Type I program (only EBNA-1). These cells are driven by the Ig/myc translocation: the role of EBV is unknown. Two further patterns, Type IIa and Type IIb, express virally encoded proteins restricted to EBNA-1 and LMP-1 or EBNA 1-6 without LMP-1 alternatively. EBV positive Hodgkin's cells express the Type IIa pattern. These cells originate in the germinal center but fail in the B cell differentiation program. Therefore, they are predestined for apoptosis but can be rescued in various ways. Infection with EBV is one of them. In vivo, they express the Type IIa program: EBNA-1, and LMP-1 and -2. It is likely that the expression of LMP-1 is induced by signals from the granulomatous microenvironment. In spite of several attempts, EBV carrying Hodgkin´s lines with the Type IIa program have not been established. The Type IIb pattern (all EBNAs but no LMP-1) has received little atten-

tion until recently. It was first seen in B-CLL cells when infected with EBV in vitro. No Type II b tumors are known. Thus, only Type III expression leads to proliferation. They represent a source of potentially malignant derivatives. However, such cells are strongly immunogenic and are kept under control unless the immune system is impaired. In healthy EBV carrier individuals the rare EBV carrying normal B cells are Type I. What is the fate of the Type IIa and Type IIb cells that are seen in IM? Under the influence of external signals, Type IIa cells may give rise to the EBV positive Hodgkin´s lymphomas. In vitro results with CLL cells infected with EBV suggest that the Type IIb cells do not survive.

NON-IMMUNE SURVEILLANCE AGAINST TUMORS

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The concept of tumor surveillance was first formulated by immunologists McFarlane Burnet and Lewis Thomas. They assumed that the immune system would recognize precancerous and cancer cells as non-self and reject them. This concept is only valid for virally transformed cells, however. In humans, EBV, HHV-8 and the papilloma viruses are relevant viral agents in this context. Tumors arising without a contribution by these viruses are regarded by the immune system as ”self”, with the possible rare exceptions like melanoma. Immunological attempts to influence them therefore imply the breaking of tolerance, a much more difficult proposition.
Multicellular organisms have powerful surveillance mechanisms of a non-immunological nature against potential neoplastic cells that threaten to disrupt the organism. Four distinct categories can be recognized: 1) DNA repair. Deficiency of repair enzymes may lead to multiple tumors and/or to multicancer syndromes. 2) Epigenetic mechanisms are currently emerging as being capable of modulating the incidence of certain tumors, e.g. by determining the stringency of imprinting and by influencing chromatin structure. 3) Intracellular surveillance. DNA damage, illegitimate activation of oncogenes, and other pathological changes may activate one or several apoptotic pathways. 4) Intercellular surveillance. The tissue microenvironment influences the probability of disseminated tumor cell growth. Moreover, appropriate differentiation inducing signals may revert the tumor cell phenotype.

MASS SPECTRAL IDENTIFICATION OF INTERACTING PROTEINS IN THE BIOSYNTHESIS OF Ni-Fe HYDROGENASES

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Mass spectrometry has become an essential tool of protein analysis in recent years. The method covers a wide range of applications from simple protein identification to the analysis of complex protein networks, characterization of post-translational modifications and *de novo* sequencing. Continuous instrumental and method development ensures improving/growing detection sensitivity, mass resolution and accuracy of the mass measurements. In the present poster we provide insight into mass spec-

trometry-based protein identification upon the maturation of the hydrogenase complex in *Thiocapsa roseopersicina*. Hydrogenases catalyze the simple redox reaction $2H^+ + 2e^- \leftrightarrow H_2$.These enzymes are classified on the basis of their metal content; some hydrogenases contain nickel and iron, while others only iron at the active center. The formation of an active NiFe hydrogenase is a very complex post-translational process requiring the concerted action of many so-called accessory proteins. The phototrophic purple sulfur bacterium *Thiocapsa roseopersicina* possesses membrane associated [NiFe] hydrogenases (HupS/L and HynS/L) and a cytoplasmic hydrogenase complex (HoxE/F/U/Y/H). Numerous specific and pleiotropic proteins involved in the biosynthesis of these hydrogenase isoenzymes were identified. The assembly of the active center is performed by sequential interaction of the accessory proteins, but the molecular mechanism of these events is not fully understood.
To identify the interacting proteins during the maturation of these NiFe hydrogenases, various accessory proteins fused to affinity tags were expressed in homologous hosts having different genetic background. The expressed proteins were purified by affinity chromatography under extremely mild conditions to preserve the molecular interactions. The components of the purified complex were then separated on SDS-PAGE and identified by mass spectrometry. The proteins were in-gel digested with trypsin and unfractionated digests were analyzed on a MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) mass spectrometer. Protein identifications based on database search of peptide mass fingerprint data were confirmed by MS-MS analyses. Using this approach, we could prove: 1) the Hox hydrogenase is a heteropentameric enzyme and 2) the HypC proteins interact with the large subunit of the Hyn and Hox enzyme. These data – supplemented with other results – suggest a special maturation mechanism for the NiFe hydrogenase in phototrophic purple bacteria.

SURVIVAL OF ASSOCIATIVE AND SYMBIOTIC NITROGEN FIXING BACTERIA IN DIFFERENT INOCULATION METHODS

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The survival of introduced bacteria is one of the main prerequisites for the success of microbial inoculations, due to the heavy loss at the environmental stress-conditions [1,2]. The loss of some nitrogen fixing bacterial strains was studied under laboratory conditions. *Sinorhizobium meliloti* (Rh-1 and Rh-2), as microsymbiont of the alfalfa (*Medicago sativa,* „Körös-1" and „Kákai legelő") and *Azospirillum brasilense* (Ab) or *Azospirillum lipoferum* (Al), associative nitrogen fixers of maize (*Zea mays,* Kiskun-4444) were used. Beside seed-surface and soil inoculations, the survival was followed in liquid broth, in a composted or in an anaerobically digested organic carrier. During the study, colony forming units of the introduced strains werefollowed by their antibiotic resistance markers, such as the kanamycin (200 µg.ml-1), and streptomycin (200 µg.ml-1) for the rhizobia or rifampicin (20 µg.ml-1) and chloramphenicol (20 µg.ml-1) for the azospirilla. Steam-sterilization of the carriers was applied at 120°C and 1.2 atm. pressure. The starting cell number of the inoculants at any inoculation was 10^6 g-1 or ml-1 unit and the loss was followed for three consecutive weeks.
No significant difference was detected regarding the surviving capacities of *S. meliloti*, *A. lipoferum* and *A. brasilense* strains in the compost and anaerobically digested organic carrier. *Sinorhizobium* strains, on the other hand, could show a better resistance against desiccation, due to their outer exopolysaccharide layers. The inoculated seed-surface supplied the most unfavourable niche for the strains. After one week of storage under laboratory conditions no *Azospirillum* colonies could be de-

tected on the surface of maize seeds. The abundance of the *Sinorhizobium* strains on the other hand was higher than 50% at the end of the experiment, with considerable differences between the used alfalfa cultivars. Among the organic carriers the best surviving ability was detected in the sterilized anaerobically digested waste. The consideration of biotic interactions should be considered when designing a new replacement of the former peat-based carriers as possible technologies.

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COMPARISON OF THE MICROBIAL COMMUNITY OF AIR-CURED AND FLUE-CURED BURLEY TOBACCOS

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Green leaves of *Nicotiana tabacum* are processed in several steps to make them suitable for consumption. The leaves are harvested sequentially as they ripen from the bottom to the top of plant. Primed leaves are usually cured in barns (light air-cured) or in drying chambers (flue-cured). The curing process involves a number of complex physical and chemical changes that occur as tobacco leaves slowly lose moisture. The final, considerable changes in the leaf properties take place during fermentation or aging. In the leaves, the changing chemical constituents and the formation of physical and aroma characteristics are strongly connected to changes in the microbial community structure of the tobacco plant. The aim of our study was to determine and to compare filamentous fungi, yeasts, and bacteria plate counts of dried Burley tobacco leaf lamina and separated stems.

Six tobacco samples were collected from three different processing sites in a Hungarian fermentation factory. Overall, a total of 149 (76 bacteria, 51 filamentous fungi and 22 yeasts) strains were isolated using a variety of selective media. The most frequently isolated bacteria were *Pseudomonas fluorescens* and *Chryseomonas luteola* species. The majority of moulds belonged to the *Aspergillus flavus* and *Cladosporium cladosporoides* species. Among the isolated 22 yeasts strains, the predominant species were *Rhodotorula minuta* and *Rh. mucilaginosa*.

Analyzing the plate counts, we found significant differences between the lamina and stem samples and the samples from light air curing vs. flue curing.

PCB DEGRADING BACTERIA FROM MARINE SEDIMENTS

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The objective of this work was to study the catabolic potential of marine sediment bacteria in PCB degradation. Bacterial communities were isolated from different marine sediments collected at urban areas along the Croatian Adriatic coast (vicinity of Rijeka, Zadar and Dubrovnik). A microcosm enrichment approach was employed to isolate bacterial communities with the capability to use biphenyl as the sole carbon and energy source. Enrichment experiments were performed in sea water diluted with mineral salts medium (SM medium) and supplemented with biphenyl. The PCB degradation ac-

tivity of enriched biphenyl-degrading communities was screened in batch culture experiments using SM medium and commercial PCB mixture (PCB 50) containing 50% chlorine and congeners with two- to heptachlorobiphenyl. More detailed characterization of the isolated bacteria and the study of their catabolic activity in the transformation of PCBs were performed with Aroclor 1248 and individual PCB congeners.
Microcosm experiments resulted in the enrichment of eight communities containing one to two members that were able to grow on biphenyl as the only carbon source. Those members, expressing 2,3-dihydroxybiphenyl dioxygenase activity were identified as species belonging to the genus *Rhodococcus* and *Sphingomonas* (by using 16S rDNA gene sequence comparison). Five out of eight enriched biphenyl-degrading communities also showed PCB transformation activity. GC/MS analyses during biodegradation experiments with Aroclor 1248 and individual PCB congeners suggested that all isolates obtained from enriched communities preferred transformation of lower chlorinated congeners (di- to tetrachlorobiphenyls) and were slightly different in their PCB transformation capabilities.

DURATION OF HPV TYPE ASSOCIATED RISK FOR HIGH-GRADE CIN IN EPITHELIAL ABNORMALITIES OF THE UTERINE CERVIX

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Previously, we have shown that the group of high-risk human papillomaviruses can be further refined by their association with high-grade cervical intraepithelial neoplasia. In the present study, we analyzed whether disease association of types 16 and 18 remains higher over time than that of the other high-risk types in a cohort of 638 patients, who had had a routine diagnostic Hybrid Capture HPV test due to squamous cell abnormalities of the uterine cervix as detected by cytology and/or colposcopy. The patients' data were followed up using the patient registry until the endpoint of histologically diagnosed *cervical intraepithelial neoplasia* (CIN).
At entry, 403 patients had cytologic atypia classified as P3 by the Papanicolau classification, 244 had a positive high-risk HPV test and 30 were infected only with low-risk HPV. During a cumulative follow-up of 16243 patient months, the cervical lesions of 200 patients were excised with 115 and 21 cases ending in histology-proven high-grade CIN and CIN1, respectively. After PCR-RFLP typing, the patients with high-risk HPV infection were analyzed in two groups: (1) types 16, 18; (2) types 31, 33, 35, 45, 51, 52, 56, 58. The type associated risk for high-grade CIN were calculated for the following periods: (1) within 12 months, (2) 13-30 months, (3) >30 months after the first detection of the cervical abnormality. The relative disease risks of type 16&18 infections were 161.4 (38.5–677.3), 196.7 (25.4–1525.2) and 29.2 (5.02–170.0) in the first, second and third observational periods, respectively. The corresponding disease risks of the other high-risk types were 47.9 (10.9–210.5), 69.7 (8.7–557.6) and zero, respectively i.e. within the high-risk group, the moderate intertype heterogeneity was maintained in the first two observational periods at least, which we suppose to reveal the prevalent and the imminent status of precancerous lesions at entry.

MICROBIAL CONTAMINATION OF VEGETABLES AND FRUITS GROWN IN HOME GARDENS

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The presence of pathogenic microbes on the surface of plant foods is generally due to faecal contamination originating from wastewater and organic fertilizers applied to the soil, the natural microflora of soil and from wild animals and insects. Therefore, eating fruits and vegetables raw, or minimally processed, might have a potential microbiological risk.
Our goal was to determine the microbial contamination of different vegetables and fruits grown in a home gardens, to trace the changes in pathogenic and spoilage microflora (mesophilic aerobic total counts, moulds and yeasts, sulphite-reducing *Clostridia, Enterobacteriaceae*, *Salmonellae*, *Listeria monocytogenes*, coliforms, *E. coli*, *Bacillus cereus*) in relation to the application of organic fertilizer (commercially available composted cattle manure). Adhesion and removal of bacteria to and from plant surfaces was also studied.
The microbial count of the soil increased with the addition of fertilizer. *Bacillus cereus* was below the detection limit in both the untreated soil and the fertilizer, but appeared in the fertilized soil. There was a correlation between the microbial load of the soil and that of green lettuce. The microbial contamination of red currant decreased with the increasing distance of berries from the soil surface. The number of sulphite-reducing *Clostridia* of spinach was higher in spinach than in the soil.
The number of pathogenic and spoilage microbes present on the surface of strawberries could be reduced with 1-1.5 orders of magnitude by washing. The mathematical statistics indicated that more data are necessary to confirm the tendencies. This work is intended to be the starting point of getting a clear picture about the microbial load of plant foods grown in fertilized soil, and the possibilities of microbes with food safety relevance entering the food chain.
Acknowledgement: The research was financed by the Széchenyi project (NKFP-4/0028/2002).

MAPPING OF DNASE I HYPERSENSITIVE SITES (HS) IN THE PUTATIVE LOCUS CONTROL REGION OF LATENT EPSTEIN-BARR VIRUS GENOMES

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The latent, episomal EBV genomes are anchored to the nuclear matrix via a putative locus control region (LCR). This region is located within the BamHI C fragment of the EBV genome, and extends from the 5' end of EBER 1 and 2 transcription units to the 3' end of the latent origin of virus replication, oriP. LCRs of cellular genes control the activity of adjacent promoters and are characterized by DNase I hypersensitive sites (HS), where ubiquitous and lineage-specific transcription factors can bind. Well characterized cell lines of type I, II and type III latency carrying strictly latent EBV genomes were used in this study. Latency type I was represented by a Burkitt's lymphoma cell line Rael. For latency type II a nasopharyngeal carcinoma cell line C666 and for latency type III a lymphoblastoid cell line CB-MI-Ral-STO were used. Nuclei isolated from these cell lines were digested with increasing concentrations of DNase I and the DNA samples isolated from the nuclei were digested with different restriction enzymes and analysed by Southern-blotting with DIG-labeling. Both unique and common hypersensitive sites were observed. The significance of these results will be discussed.

CYTOTOXICITY SCREENING OF LOW-MOLECULAR-WEIGHT METABOLITES OF *CANDIDA* SPP.

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Opportunistic yeast-like fungi of *Candida spp*. are still the main fungal isolates, especially in the case of debilitated, immunocompromised patients, patients under solid-organ transplantation, etc. With the exception of the host factors, which promote invasiveness of commensal fungal flora, several virulence factors can contribute to the pathogenicity of mycoses caused by *Candida spp*. These factors are: polymorphism and phenotypic switching, secretion of hydrolytic enzymes, such as proteinases, phospholipases, hemolysins. The expression of these factors differs among species, and even between strains. Our recent studies showed that during exponential growth in vitro of several species of clinical *Candida spp*., almost all strains secreted low-molecular-weight metabolite (LMW) 3-indoleethanol (syn. tryptophol as the end product in tryptophan catabolism). We did not identify secretion of a highly toxic LMW metabolite gliotoxin, the production of which by *C. albicans* is probably controversial, since gene cluster involved in the gliotoxin biosynthesis pathway was identified in *A. fumigatus* genome, although *C. albicans* does not contain such a cluster [1].
The aim of this study was to screen cytotoxicity of tryptophol and its precursor 3-indolelactic acid, together with gliotoxin using MTT colorimetric assay. The screening was evaluated on five cell lines from the ECACC cells archive: human monocytic leukemia (THP-1, monocyte), human lung carcinoma (A549, epithelial), human Caucasian hepatocyte carcinoma (Hep G2, epithelial), Chinese hamster ovary (CHO, epithelial) and African green monkey kidney (COS-7, fibroblast), and the results were expressed as IC50. Gliotoxin showed 1000x lower IC50 than tryptophol and 3-indolelactic acid, whereas IC50s were between 2 and 11 μM, with the THP-1 and A549 being the most sensitive. Tryptophol showed IC50 concentration between 2 and 7 mM, with the lowest IC50 value on the THP-1 cell line. 3-indolelactic acid had 2 or 3 times higher IC50 values (between 4 and 9 mM), indicating a lower cytotoxic potential than tryptophol.
The next step in our research is to evaluate the in vivo secretion of these LMW metabolites during mycoses caused by *Candida spp*. as possible virulence factor.

[1] Gardiner DM et al.: Microbiology **151**, 1021-1032 (2005).

STUDY OF THE DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI – UNIQUE LINEAGES OR ONLY THE LACK OF INFORMATION?

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Arbuscular mycorrhiza (AM) is the most abundant mutualistic plant-fungus interaction type, which is formed by a monophyletic group of fungi, the Glomeromycota. While the species diversity of this old

lineage is relatively low, the fungi belonging to this phylum show remarkable genetic polymorphism e.g. in the nrRNA gene sequences.
During the work presented here we studied the molecular diversity of AMF colonizing the roots of fern *Botrychium virginianum* and gymnosperm shrub *Ephedra distachia*. Semi-nested PCRs were carried out with primer pairs specific to different groups of Glomeromycota. One set of the primers targeted the partial SSU and ITS of the nrRNA gene, while the other set was designed to amplify a part of the SSU. Both reactions amplified approximately 1 kbp long parts; the amplicons were cloned, and several clones from each reaction were sequenced. No identical sequences, including the ITS-part, were found, which result corresponds with the general observations of molecular studies of AMF. Some of the clades formed by the sequences show similarities with, while others separate well from, GenBank data of known AMF species. Due to the high variability of sequences containing the ITS region, no unambiguous alignment and thus no phylogenetic analysis could be done with species representing the whole phylum.
The partial SSU sequences together with GenBank data could be used for inferring phylogenies. Some unique clades were formed by the sequences of AMF of the two plant species. Single or no molecular information on AMF fungi of these two distinct plant groups has been presented previously, and these are the first AMF sequences from sandy areas of the Great Hungarian Plain. Although some results might be interpreted as being a specific lineage, further data on AMF of these areas are necessary to draw unambiguous conclusions. However, some data already promise very interesting outcome about the ecology of either the fungi or the plants of the studied interactions.
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HUMAN CYTOMEGALOVIRUS STRAINS ELICIT DIFFERENT LEVELS OF IL-8 AND PERMISSIVENESS IN EPITHELIAL CELLS

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Epithelial cells are targets of permissive infection by human cytomegalovirus (HCMV) in vivo during acute disease. However, studies on HCMV-epithelial cell interactions in vitro have furnished discordant results. In a previous study, we observed that syncytiotrophoblast (ST) cells infected with HCMV strains isolated from congenital infections induced different amount of interleukin-8 (IL-8) and immediate-early (IE) viral gene expression. This suggests that certain HCMV strains induce a high level of IL-8 and augment their replication in ST cells, whereas others can replicate if IL-8 is provided by coinfection with other viruses or bacteria. It also indicates that interstrain differences in HCMV-epithelial cell tropism may determine the outcome of HCMV infection. To test this hypothesis and to establish whether the permissiveness of HCMV infection in epithelial cells depends primarily on the IL-8-inducing capacity of the strain, A549 cells (human lung alveolar epithelial cells) were infected with HCMV isolates of different origins. The relationships between the amount of IL-8 produced by the infected A549 cells, the number of nuclei of cells expressing the CMV IE gene and the release of infectious virus were examined. The data revealed that the IL-8-inducing capacities of the various HCMV isolates differed in the A549 cell cultures, and the IE gene expression and the virus production were IL-8 dose-dependent. Infection by one of the HCMV isolates (128V) progressed to complete lysis of an inoculated A549 monolayer. These results confirmed that HCMV replication in epithelial cells is strain-dependent, suggesting that specific viral gene(s), including UL146, which encodes a potent CXC chemokine similar to IL-8 , are required for efficient replication in this cell type.

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PRODUCTION OF ENZYMES BY SOLID-STATE FERMENTATION

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Solid-State Fermentation (SSF) is defined as the growth of microorganisms on solid materials in absence (or near absence) of free water. However, the substrate particles must contain enough moisture to support the growth and metabolism of the microbes. SSF is a complex heterogeneous three-phase (gas-liquid-solid) system, which occurs widely and spontaneously in nature. The concept of using solid substrate is probably the oldest method used by man to make microorganisms work for him. SSF provides numerous opportunities in the production of microbial enzymes, since a large number of microorganisms and various agro-industrial residues can be used in the process. Solid-state fermentation offers the greatest possibilities when fungi are used.

Acknowledgement: Joint research on a-amylase, L-leucine-aminopeptidase, cellulase, chitinase, lipase, phytase and xylanase enzymes with partners in India, USA, South Africa and Malaysia by SSF is discussed.

RELATIONSHIP BETWEEN HYDROPHOBICITY AND FILM-FORMATION OF *SACCHAROMYCES CEREVISIAE* ON LIQUID

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Biofilm forming *Saccharomyces cerevisiae* strains are able to lift up to the surface of liquids and form pellicle on culture media containing non-fermentable carbon-sources. Film formation is influenced by several environmental factors but their exact identification is still lacking. The role of different cell surface molecules is not clarified at all.

We compared cell surface hydrophobicity of film-forming and non-film-forming *Saccharomyces cerevisiae* strains at different growth phases and studied the effects of pH, sugars, metal ions, proteases and a cell wall lytic enzyme on the hydrophobicity of cells.

Our results indicated that film-forming strains are strongly hydrophobic at acidic pHs at any phase of growth, while they are hydrophilic in the alkaline range. Optimum of film formation is between pH3 and 4. In contrast to this, non-film-forming strains are hydrophilic at any pH ranges.

We might suppose that Ca-dependent lectin molecules at the cell surface attach to the carbohydrate moieties of the cell wall during film formation, similarly as in the case of another well known type of cell aggregation, flocculation. Therefore we studied the effect of different mono-and disaccharides containing glucose, mannose, fructose and galactose monomers on the disintegration of biofilm but none of them showed any effect. Integrity of the biofilm was not influenced by chelation of Ca-ions with EDTA-Na2. These results indicate that not the lectin-type proteins are responsible for the cell-

to-cell attachment in the biofilm. Certain proteases like proteinase K and pronase and the cell wall lytic enzyme, lyticase strongly reduced the hydrophobic character of cells.
We studied the hydrophobicity of a series of mutants lacking one or more cell wall proteins aiming to know whether any of them had a role in cell surface hydrophobicity. We found that none of them had a crucial role in the hydrophobic or hydrophilic characters of cells walls.

MICROBIAL CHANGES DURING THE RIPENING OF CROATIAN NATURALLY FERMENTED SAUSAGE

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Fermentation is a well-known method of conservation and of extending the shelf life of meat. Fermentation, ripening and drying processes of dry fermented sausages are characterized by intensive microbial and physicochemical changes that have a great impact on the quality and safety of the final products. The aim of our study was to determine the changes of "wild" microbial flora during the processing of Croatian naturally fermented sausage. Three batches of sausages were used for the experiments. Samples were taken at 0, 2, 4, 7, 14 and 28 days after formulation, transported to the laboratory and subjected to microbiological analysis. Total viable count (TVC), lactic acid bacteria (LAB), micrococci, enterococci, enterobacteria, pathogenic staphylococci, yeasts and moulds, *Psudomonas* spp., *Salmonella* spp. and *Listeria monocytogenes* were detected using classical microbial methods (ISO). Furthermore, a total of 150 strains of LAB and 72 strains of catalase-positive cocci were isolated and characterized biochemically using API 50 CHL and API Staph, respectively. The total viable count and LAB count were quite similar after the 4th day of fermentation, but at the end of ripening, the total viable count was lower than the LAB count. In spite of that, the initial LAB population was low in batch 1 (4 log10 cfu g^{-1}) and from batch 2 and 3 (day 0) LABs were not isolated. During ripening, lactic acid bacteria became the dominant microbial flora (7-8 log10 cfu g^{-1} at the 28th day). The number of micrococci in the sausages during fermentation was very low (2-3 log10 cfu g^{-1} at the end of ripening). This observation reflects their poor competitiveness in the presence of lactic acid bacteria and other influences during fermentation. The number of pathogenic staphylococci as well as enterobacteria was lower than 2 log10 cfu g^{-1} after stuffing, while neither staphylococci nor enterobacteria was detected on the 7th day of fermentation. Yeasts were isolated from day 0 to day 14 and their number decreased during fermentation and finally on the 28th day they were not detected in the sausages. Enterococci, *Pseudomonas* spp., *Salmonella* spp. and *Listeria monocytogenes* were not found in the sausages during fermentation, ripening and drying of sausages. Biochemical determinations of LAB isolates showed that the prevalent microorganisms in Croatian naturally fermented sausage were lactobacilli (133 isolates, 88.6%). Among them, the dominant species was *Lactobacillus plantarum* (51 strains, 34%). Leuconostoc, lactococci and pediococci were also determined. Among 72 catalase-positive cocci, all of which were staphylococci: *Staphylococcus xylosus* (21 isolates, 29.2%), *Staphylococcus capitis* (18 isolates, 25%), *Staphylococcus carnosus* (18 isolates, 25%), *Staphylococcus saprophyticus* (15 isolates, 20.8%).

GENOMIC INVESTIGATION OF AN UNKNOWN HONEY BEE (*APIS MELLIFERA* L.) VIRUS FOUND IN HUNGARY

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During the etiological investigations carried out on samples collected in an apiary experiencing severe losses, viruses were demonstrated. The virus was identified as an acute bee paralysis virus (ABPV) by electron microscopic (EM) investigations and agar gel immunodiffusion (AGID) tests used in the identification of bee pathogenic viruses. Since neither the EM nor the AGID is specific enough to differentiate the morphologically similar and antigenically related viruses, and multiple infection could not be excluded in the samples, molecular test methods were worked out to detect ABPV and its variant, Kashmir bee virus (KBV), in the samples. Following reverse transcription (RT-PCR) polymerase chain reactions were applied to amplify certain regions of the positive single stranded genomes of the viruses by using oligonucleotide primer pairs designed upon the sequences of ABPV and KBV deposited in the international gene bank, and the products were sequenced. The sequences were aligned and compared to the sequences deposited in the gene bank. Some amplicons produced by the different primers had shown similarity to ABPV, others to KBV sequences. The results may be explained by the presence of at least two morphologically undistinguishable and antigenically and genetically related viruses in the samples. Comparative studies of other genomic regions revealed that one of the viruses present in the sample is a Central European strain of the ABPV showing 93% identity to the reference strain. The other viral component can be identified neither as ABPV nor as KBV. Although the sequences of the new virus show similarity (60-75%) on certain regions to KBV, other parts of the sequence show similarity (60-70%) to ABPV. Following $CsCl_2$ gradient ultracentrifugation, virus isolation in bee pupae was performed and KBV specific primers were made for further examinations. As a result we sequenced a 3094 bp long part of the new virus. Further investigations are necessary to clear the phylogenetic position and the taxonomic status of the new virus.

APPLICATION OF RAPID SUBTRACTION HYBRIDIZATION FOR THE STUDY OF OPPORTUNISTIC PATHOGENICITY RELATED GENE EXPRESSION IN *TRICHODERMA LONGIBRACHIATUM*

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Certain groups of filamentous fungi are of increasing clinical importance as emerging opportunistic pathogens of humans. Species belonging to the filamentous fungal genus *Trichoderma* are well known as potential candidates for the biological control of plant pathogenic fungi and as cellulase producers of biotechnological importance. On the other hand, they are also on the growing list of potential fungal pathogens in immunocompromised hosts. About 50 clinical cases are known with the involvement of *Trichoderma* strains, most of them belonging to species *T. longibrachiatum*. Supposed virulence factors of *T. longibrachiatum* as an opportunistic pathogen include the ability of growth at 37 ºC and physiological pH, the production of extracellular proteases and toxicity to mammalian cells. A strategy for the study of these and other possible virulence factors is the identification of genes that show pathogenicity-related expression. An experimental system of simulated infection

was therefore developed and the method of Rapid Subtraction Hybridization (RaSH) was used for the detection of genes ifferentially expressed between a clinical *T. longibrachiatum* isolate that proved to be toxic to mammalian cells in previous studies, and a non-clinical, non-toxic isolate belonging to the same species.
Culturing experiments at 37ºC revealed that *Trichoderma* strains are capable of growth in RPMI and BEGM (bronchial epithelial growth medium) media used for culturing of human cell lines. Simulated infection experiments were performed by inoculating monolayer cultures of human bronchial epithelial cells in BEGM medium with different concentrations of *Trichoderma* conidial suspensions. The cell cultures showed extreme susceptibility when inoculated with an end concentration of 10^6 conidia/ml. The human cells died within a few hours. In the case of an inoculation with 10^5 conidia/ml, living human cells could be detected even after an incubation time of 24 hours in case of co-cultivation with non clinical, non toxic strains, in spite of the presence of the intensively growing fungus. In contrast, co-cultivation with toxic clinical strains again resulted in the death of the human cells within less than 24 hours. *Trichoderma* mycelia were harvested and subjected to total RNA extraction, which was followed by the RaSH experiment performed between a clinical, toxic isolate as the tester and a non-clinical, non toxic one as the driver. The application of this approach revealed a subtracted plasmid library containing sequences differentially expressed in the clinical, toxic *T. longibrachiatum* isolate. The experimental system and the progresses of the project are presented.
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INCREASING FREQUENCY OF MRSA CARRIAGE IN UPPER RESPIRATORY TRACT OF PATIENTS

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MRSA carriage of patients being admitted to a hospital is of outmost importance in generating colonisation and/or infection of other patients with such microorganisms.
The incidence of both the MRSA and MSSA carriage in the nares and the throat, respectively, of 2039 patients admitted to the hospitals of the Semmelweis University between January 2004 and July 2005, was examined.
Samples from the nares and the throat of each patient were obtained with swabs of Biotest Transport System and subcultured on blood agar and in 1% glucose-meat Mueller-Hinton broth as enrichment medium, and incubated overnight at 35°C. *Staphylococcus aureus* strains were identified by conventional methods including rapid slide agglutination (Bio-Rad). When necessary, confirmation was done by detecting the presence of *S.aureus* specific nucA gene by polymerase chain reaction (PCR). MRSA was screened on 6 mg/L oxacillin agar plate, and MIC of oxacillin for the strains was determined with the broth microdilution method according to the guidelines of the NCCLS. Methicillin resistance was confirmed by PCR amplification of the mecA gene. MRSA strains were genotyped by pulsed-field gel electrophoresis (PFGE) using SmaI.
Twenty percent of the patients carried *S.aureus* in their nose and throat. 7.5% and 4.4 % of strains isolated from the nares and the throat proved to be MRSA, respectively. A considerable increase was seen in the incidence of MRSA carriage from 2004 to 2005. Extrapolating the results to the total number of patients, 1.5% was MRSA carrier in the nose and 0.9% in the throat. PFGE patterns revealed three main different MRSA clones.
MRSA strains are present in the upper respiratory tract of patients with increasing frequency in our area, therefore, screening for such strains at the time of hospital admission has to be considered.

TRICHODERMA: A VERSATILE INDUSTRIAL FUNGUS GOES GENOMIC

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The genus *Trichoderma* (Hypocrea) contains several fungi of industrial interest, such as *Trichoderma reesei*, which is a producer of cellulases and recombinant proteins, and *T. harzianum*, *T. atroviride*, *T. virens* and *T. asperellum* who are potent biocontrol agents. The interest in these fungi has recently resulted in the publication of the complete genome sequence of *T. reesei* (=*Hypocrea jecorina*), and the sequencing of >10 000 ESTs from biocontrol species. In this lecture, I describe selected examples of the use of the genome information for biomining of *Trichoderma* and the use of *H. jecorina* as a "model" *Trichoderma* for understanding its physiology and application.

GLYCOSYNTHASE REACTION - AN ALTERNATIVE SYNTHESIS ROUTE FOR THE PRODUCTION OF BIOACTIVE OLIGOSACCHARIDES

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Nowadays great interest arises on carbohydrate based pharmaceuticals and the development of techniques for the synthesis and analysis of oligosaccharides. Direct chemical synthesis is often very difficult due to the need of regio- and stereo selectivity. The use of glycosynthase enzymes represents a solution to these problems. Glycosynthases are mutated glycosidases whose catalytic nucleophile amino acid changed to a non nucleophilic one causing efficient synthesis of oligosaccharides but losing the ability to hydrolase them.

In our project we focused on beta-xylo- and beta-mannooligosaccharide synthesis. For glycosynthase construction we chose the robust β-mannosidase (ManB, EC 3.2.1.25) and β-xylosidase (XynA, EC 3.2.1.37) produced by *Thermobifida fusca*, a compost inhabiting thermophilic microbe. Earlier we described the β-mannosidase as a retaining GH2 family enzyme, while the β-xylosidase is an undescribed member of the GH43 family. Both enzymes showed remarkable transferase activities.

In the presence of para-nitrophenyl β-D-mannopyranoside (pNP-βM) and pNP-αM, substrate mannosyl groups were transferred by ManB from pNP-βM to pNP-αM, resulting in the synthesis of disaccharides in an amount of 1 to 2%.

The transferase activity studies of XynA measured by HPLC revealed that the enzyme shows remarkable transferase activity with xylobiose and pNP-β-D-xylopiranoside as donor and pAP-1-thio-β-D-xylopiranoside as acceptor, as products of a higher polymerization degree form.

Based upon recent success with a GH family 2 glycosynthase, we obtained a mannosynthase from ManB by replacing the catalytic nucleophile Glu530 with serine by site directed mutagenesis. The tentative assignment of Glu530 as the acid-base catalyst of ManB was confirmed by the total loss of hydrolase activity observed for the mutant Glu530Ser enzyme in the presence of xylobiose and pNP-βM substrates. The mutant enzyme proved to be a much more effective glycosynthase then the wild type ManB, since with the use of α-mannosyl-fluoride as donor molecule, the amount of oligosaccharides formed was one order of magnitude higher. We do believe that the glycosynthase approach will be applied successfully to the large–scale production of bioactive oligosaccharides in the near future.

CLONING AND BIOCHEMICAL CHARACTERIZATION OF A B-XYLOSIDASE (XYNA) A NEW MEMBER OF THE HEMICELLULASE ENZYME SYSTEM OF *THERMOBIFIDA FUSCA*

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We cloned a gene encoding a β-xylosidase enzyme (EC 3.2.1.37) from *Thermobifida fusca* strainTM51 by the use of an expression library constructed in *Streptomyces lividans* strain TK24.
For screening the recombinant *S. lividans* host clones, we used the fluorogenic substrate methylumbelliferyl-b-D-xyloside (MUX). From the 2000 thiostreptone resistant clones, we found one xylosidase producing colony. We subcloned and sequenced the beta-xylosidase gene, which is 100% identical to the sequence of the putative xylosidase of *T. fusca* YX annotated by the genome project.
The enzyme (XynA) belongs to the inverting glycosyl hydrolase (GH) 43 family. The xylosidase (XynA) of *T. fusca* is an intracellular 62 kDa enzyme with a pI of 5.45. We could detect the enzyme at a size of 130 kDa after SDS-PAGE by activity staining using MUX substrate. It seems that b-xylosidase is a homo-dimeric enzyme, as after heat shock treatment it dissociated into two equal 62 kDa monomers accompanied by the total loss of enzyme activity measured by zymography.
The temperature and pH-dependence of activity of β-D–xylosidase were also determined. The highest activity of the xylosidase was at 50°C. The pH optimum of activity was at around 7.0 in 0.1 M phosphate buffer. We measured the substrate specificity of the xylosidase on xylooliogomers. Xylobiose (X2) and xilotriose (X3) were hydrolyzed by the enzyme while with the use of the X4 substrate in an independent experiment, we could measure no hydrolase activity indicating the X2-X3 preference of the enzyme. 1-thio-methyl-β-xylopiranoside was found to be a competitive inhibitor of the enzyme (Ki=1,27 mM). According to the preliminary results of synergistic tests with different xylanases of *T. fusca* on different xylan substrates, it seems that beta-xylosidase is a component of the xylanase system of this bacterium. The addition of purified XynA to endoxylanases and acetyl xylan esterase increased the hydrolysis of xylan, suggesting a truly synergistic relationship. Besides creating xylose for metabolism, the role of this enzyme might be unlocking the feed-back inhibition of the xylanases by hydrolyzing xylobiose.

ISOLATION AND TAXONOMIC INVESTIGATION OF A STABLE CELLULOLYTIC CO-CULTURE FROM MESOPHILIC COMPOST

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Using a special enrichment technique, a mixed cellulolytic isolate was gained from a mesophilic compost environment. Any attempt to achieve stable pure cultures from the original isolate with se-

rial dilution techniques (also by the use of Potter-homogenizer; most different media) failed and it has been realized that the isolate is a stable co-culture of two Gram-negative bacteria. Strain K07 is the dominant component of the co-culture as this strain in pure culture produced a series of endoglucanases, exoglucanases and cellobiase beside endomannanases, thus grows well on highly crystalline MN300 cellulose carbon source, while strain KSz grows moderately on cellulose and shows only weak endoglucanase activity. The differences between the two strains manifested themselves in classical-morphological and in molecular traits. By the use of transmission electron microscopy, negatively stained preparations of strain KSz showed cells with a polar flagellum and inclusion bodies were found, compared to the peritrichous flagellation of strain K07. The carbon source preference of the isolates differed significantly, measured by 30 different saccharides. Based on the results of physiological characterisation, both strains are "upper-mesophilic" microbes with a 40°C temperature optimum. Phylogenetic analysis of strain K07, based on 16S rRNA gene (1469 bp) sequence comparison, revealed that the isolate was affiliated to the gamma subdivision of *Proteobacteria*. However, the homology values were quite low. The highest similarities were detected with *Pseudomonas cellulosa* (91%), *Teredinibacter turnerae* (90%), *Cellvibrio mixtus* (90%), *Pseudomonas aeruginosa* (89%) and *Pelagiobacter variabilis* (89%), respectively. Comparison of 16S rRNA (1443 bp) gene sequences showed that strain KSz fell within the radiation of the cluster comprising *Aquabacterium* species. The highest 16S rRNA gene sequence similarities were found with *Aquabacterium citratiphilum* (97.4 %), *Aquabacterium communei* (97.1 %) and *Aquabacterium parvum* (96.3 %).
The most interesting finding was the ability of strain K07 to grow on Whatman No.1. filter paper forming fruiting body, which is a unique trait in the gamma subdivision of *Proteobacteria*. The 16S rDNA analysis based taxonomic characterization of the strains predicts the possibility of new taxa. Most possibly a new genus should be created for the placement of strain K07.

CLONING AND PURIFICATION OF THERMOSTABLE HYDROLASES OF *THERMOBIFIDA FUSCA* STRAIN TM51 BY GENOME MINING

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Members of the genus *Thermobifida* comprise one of the most active groups of lignocellulose decomposing compost inhabiting bacteria. These actinomycetes produce multiple cellulases, xylanases and lignolytic enzymes belonging to different glycosil hydrolase families (GH). While there are abundant data on the cellulolytic system of *T. fusca*, the hemicellulolytic enzyme system of this species is still poorly characterized. Using the recently available results of the genome project of *T. fusca*, putative endoxylanases, acetyl xylan esterase and endomannanase were identified in the genome of this thermophilic actinomycete. To obtain a deeper insight into the hemicellulolytic enzyme system of *T. fusca,* we prepared fusion protein constructs of these unknown hemicellulases in addition to the biotechnologically important known thermostable cellulase, xylanase, mannosidase and xylosidase enzymes. For heterologous expression of the respective proteins in *Escherichia coli,* we cloned the genes into pET-28 vector following routine procedures. For the gradient PCR reaction, we used genomic DNA template from *T. fusca* strain TM51. *E. coli* BL21 (DE3) competent cells were transformed with the plasmid constructs. The expression of His-fusion proteins were induced with IPTG and further purified by Ni-NTA affinity chromatography. For identification of the expressed hydrolases we employed zymography, and activity tests with fluorogenic substrates or Remazol-

Brilliant-Blue coupled substrates. During the past year we successfully cloned and purified the following hydrolases by the use of Pharmacia Akta HPLC system: endoglucanase Cel5B (EC 3.2.1.4, endo-1,-4-β-glucanase, GH5 family), β-xylosidase XynA (EC 3.2.1.37, GH43 family), endoxylanase TfXA. (EC 3.2.1.8, endo-1,-4-β-xylanase, GH11 family; with C terminal cellulose binding module – CBM-2 domain), endoxylanase XylA (EC 3.2.1.8, GH10 family), endoxylanase XylB (EC 3.2.1.8, GH10 family), acetyl xylan esterase AxeA (EC 3.1.1.72, Carbohydrate Esterase –CE- 7 family; with C terminal CBM2 domain), β-mannosidase ManB (EC 3.2.1.25, GH2 family), endomannanase ManA (EC 3.2.1.78, mannan endo-1,4- β-mannosidase, GH5 family). The yield of the expressed proteins was in the range of 70-150 mg/l. These results warrant further use of the enzyme constructs both in basic- (biochemical characterization of the new hydrolases, crystallography) and in applied-research (e.g. in oligosaccharide synthesis or using the enzymes as feed additives).

EFFECT OF ANTIBIOTIC TREATMENT ON BACTERIAL ADHERENCE TO ACRYLIC INTRAOCULAR LENSES

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Postoperative endophthalmitis (POE) is a serious and damaging complication of intraocular surgery. It is a rare but serious inflammation that may arise from any surgical procedure that disrupts the integrity of the globe. POE might have severe consequences, such as visual loss or retinal detachment. The sources of bacteria that cause endophthalmitis are the periocular skin and eyelashes. By far coagulase-negative *S. epidermidis* and *S. aureus* are the most frequent etiological agents in acute POE. Implantation of artificial intraocular lenses presents an abiotic surface to which these bacteria may attach and form biofilm. Antibiotic therapy is usually ineffective because of the poor penetration of antibiotics into the adherent biofilm, and because of the changed properties and reduced multiplication rate of bacteria in the biofilm matrix.

In this study the adhesive ability of 7 *S. aureus* and 11 coagulase-negative *Staphylococcus* (CNS) strains to the surface of acrylic intraocular lenses was examined by ultrasonic method. In untreated cases, adhesion of *S. aureus* and CNS strains did not differ significantly. In this study a single – 60-minute-long - antibiotic (ciprofloxacin and tobramycin) treatment had been applied, which correlated well with the single or intermittent antibiotic prophylaxis of patients. Ciprofloxacin administration was able to significantly reduce the number of attached cells on the surface of acrylic lenses both in the case of *S. aureus* and CNS strains. Dependence of the effect on concentration could also be demonstrated. Tobramycin treatment was able to significantly inhibit only the attachment of the *S. aureus* cells. Antibiotic treatment can induce changes in the function and morphology of bacterial cells, which might contribute to the altered adhesive properties. Despite the debate on antibiotic prophylaxis we presented that a single antibiotic administration can decrease the attachment of bacterial cells to the surface of acrylic lenses and might be effective in the prevention of POE.

DEGRADATION OF THE FIBRE COMPONENTS OF WHEAT STRAW AND MAIZE STALK BY THE THERMOPHILIC FUNGUS *THERMOMYCES LANUGINOSUS*

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The enzyme-producing capacity of different substrains of the fungus *Thermomyces lanuginosus* NCAIM 001288 was studied under experimental conditions *in vitro*. All five substrains studied were found to possess adequate xylanase activity (225–245 U/ml). Although, the cellulase activity of the strains was low (3 U/ml), the cellulolytic ability of the cultures could be clearly demonstrated. Based upon the results of maize stalk and wheat straw decomposition tests, the fungus is also likely to have ligninase activity, and its pectinase activity was also demonstrated. Under sterile conditions, *T. lanuginosus* has a high fibrolytic activity, while under non-sterile conditions it has to compete with the original fungal biota present on plant materials, in order to exert its effect, thus only lower enzyme activity values could be measured. On the basis of our results, the *T. lanuginosus* strain studied was capable of degrading wheat straw and maize stalk, thus, following further studies, it may be suitable for the supplementation of ruminant feed.

CHARACTERISATION OF A NOVEL MITOCHONDRIAL PLASMID IN *FUSARIUM PROLIFERATUM*

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A 10.3 kb linear mitochondrial DNA plasmid was isolated from *Fusarium proliferatum* (*Gibberella intermedia*) strain ITEM 2336 and designated pFP1. Cleavage by proteinase K and exonucleases indicated that the 5' end of pFP1 DNA was associated with a terminal protein. Sequencing the entire pFP1 DNA revealed that the plasmid consists of 10 336 bp with perfect terminal inverted repeat (TIR) sequences of 400 bp. Two major, non-overlapping ORFs were identified on opposite strands, encoding a phage-type RNA polymerase (ORF1) and a family B type DNA polymerase (ORF2), respectively. Limited sequence homology suggested that the terminal protein is encoded by the N-terminal domain of ORF2. One additional minor ORF was also detected with partial homology to mORF1 of the pMLP1 plasmid from the white-rot fungus, *Pleurotus oestreatus*. Similarly to mORF1 of *P. oestreatus*, this minor ORF of pFP1 encodes a highly basic protein (pI = 9.25), acting most likely as a TIR-binding protein. Minor ORF of pFP1 shared a common transcript with ORF1. Primer pairs designed to detect pFP1 in a wide range of fungi belonging to *Gibberella fujikuroi, sensu lato* confirmed the presence of the plasmid in other four isolates of *F. proliferatum*. Real-time experiments revealed 0.6-3.4 copies of plasmid relative to the mtDNA copies. In the case of isolates ITEM 2336 and ITEM 2343 ethidium bromide treatment was used to cure the plasmid. Real-time PCR analysis of a total of 250 surviving cultures indicated that no plasmid-free strains could be obtained by this treatment. Moreover, significant reduction of the copy number of the plasmid was only detected in three surviving clones of ITEM 2343.

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PORCINE CIRCOVIRUS TYPE 2 INFECTION IN WILD BOAR (SUS SCROFA) IN CROATIA

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Porcine circovirus type 2 (PCV2) infection in Croatian wild boars (*Sus scrofa*), associated with morphological lesions is described, which are regarded as characteristic for postweaning multisystemic wasting syndrome (PMWS) in domestic pigs. During the hunting season of 2003/2004, eight wild boars – approximately 2-6 months of age – were found dead in a fenced hunting area and were necropsied at the Pathology Department in Croatian Veterinary Institute. The diagnosis of PMWS met all three key criteria: the presence of compatible clinical signs of wasting, the presence of the characteristic microscopic lymphoid lesions, and the detection of PCV2 within the lesions by in situ hybridisation (ISH). The PCV2 DNA from swine tissues was extracted and sequenced. The phylogenetic analysis indicated the distribution of only one PCV2 strain in wild boars in Croatia, which was genetically almost identical with PCV2 isolated in France, the Netherlands, United Kingdom, China and Slovenia.

EARLY CYTOLOGICAL EVENTS IN CASE OF ATYPICAL INCOMPATIBLE RELATIONSHIP BETWEEN VIRUS AND PLANT

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In the present work, we have studied the early symptoms and cytological characteristics of the atypical incompatible relationship between *Obuda pepper* virus (ObPV) and *Nicotiana tabacum cv. Xanthi nc.* During the experiments, we have used local inoculation method combined with temperature shift (TS) to detect and localize the real infection sites. According to the literature, we expected the collapse of the whole infected region because of the hypersensitive response (HR). Still we found a surprising phenomenon. In a small population of the inoculation points chlorotic spots appeared and local lesions could only be observed around these areas, forming a ring. We supposed that this ring-shaped area may border the infected region, and based on electron microscopy studies, we found this hypothesis valid. Outside the chlorotic area and the HR-ring the cells are intact and are in good accordance with the control. This fact suggests that the plant is able to sense the boundary between the healthy and infected tissues, and properly induces programmed cell death (PCD) there. Fine structure of the chlorotic region, the HR-ring, the neighboring tissues and the higher leaves were examined in early moments after the TS. Similarly to certain stressed tissues, high amount of starch, degradation of plastids, division of mitochondria and altered pattern of chromatin condensation were very characteristic in the HR region. The relation between chlorosis and appearance of lesions seems to be unambiguous, however we can not explain the lack of chlorotic regions at other infection points. Our observations of this phenomenon do not show any correlation with the quantity of inoculum or age of the leaves. On the basis of background information, we suspect that there may be a yet unknown factor in HR initiation, which can be a histological unit, for example the bundle sheath. Investigations on ObPV inoculation with and without TS show similar tendencies in point of systemization. Serious degradation of vascular bundle sheath and mesophyllar cells can be observed in the higher leaves with electron microscope when virions enter the phloem but not yet released from it. It might be caused by a signal originating from the vascular system, because the wave of degradation proceeds from the cells surrounding the vascular bundle to the distant tissue elements.

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DETECTION OF UROGENITAL MYCOPLASMAS FROM HEALTHY CHILDREN AND CHILDREN WITH URINARY TRACT INFECTION

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Detection of urogenital mycoplasmas from urinary tract infection (UTI) of children, and from urine of healthy children, using a commercially available kit (Mycofast Evolution2 and 3, International Microbio). Our study included 168 children presenting symptoms of UTI, with repeatedly negative urocultures. The other group consisted of 100 children with no signs of UTI. The culturing of mycoplasmas and the antibiotic susceptibility testing (doxycycline, roxythromycine, ofloxacine) were performed using the broth and various substrates provided by the kit in a gallery system.
From the 168 cases, 44 were positive for urogenital mycoplasmas, 38 strains of *Ureaplasma urealyticum* and 23 strains of *Mycoplasma hominis* were identified. In 18 cases, the two species were simultaneously present. The susceptibility pattern of the isolates was diverse. From the 100 urine probes collected from children with no signs of UTI, 6 were positive for urogenital mycoplasmas. *M. hominis* and *U. ureaplasma* might be detected from children's UTI. The colonisation of healthy children with urogenital mycoplasmas was significantly lower.

VECTOR FOR TUMOR GENE THERAPY THROUGH INHIBITION OF ANGIOGENESIS: ADENOVIRUSES BEARING NGR MOTIFS IN THE HI-LOOP OF ADENOVIRUS FIBER PROTEIN BIND AMINOPEPTIDASE N AND ALPHA V BETA 3 INTEGRIN

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Genetic retargeting of adenovirus type 5 (Ad) through insertion of sequences in the fiber protein should permit targeted gene delivery. The APN (aminopeptidase N) expressed on the endothelial cells of angiogenic vasculature, represents a target molecule for tumor gene therapy aimed at inhibition of angiogenesis. It has been shown by phage display that the motif NGR (asparagine-glycine-arginine) binds to APN.
The aim of this work was to design a vector retargeted on the APN molecule via incorporation of specific NGR-containing ligands into the HI loop of a fiber protein, and to examine the effect of linear and cyclic sequences on its targeting properties. We constructed four replication defective adenoviruses bearing cyclic or linear NGR-containing sequences. These insertions did not affect structure or incorporation of the fiber protein in viral particles. We have shown on the rhabdomyosarcoma (RD) cell line, which expresses APN but only at very low levels of avb3 integrin and coxsackie-adenovirus receptor, that all NGR-bearing adenoviruses exhibited moderately increased transduction efficacy in comparison with the wild type virus. NGR-bearing adenoviruses containing cyclic motifs were more

efficient than those containing linear ones. The increased transduction efficacy of NGR-bearing Ads was completely abolished by the APN-specific peptide CNGRC and the integrin-specific peptide CRGDC. By measuring transduction efficacy on human laryngeal carcinoma cells with graded expression of avb3 integrin, we found that NGR-bearing viruses bound weakly to this integrin. Nevertheless, adenoviruses bearing linear motifs were more efficient than those bearing cyclic NGR. Additional evidence that the improved entry of NGR-bearing Ads into RD cells was mediated by binding to APN, and perhaps to avb3 integrin as well, was provided by experiments in which RD cells were treated with TGF-b1. Such treatment, which up-regulated APN and avb3 integrin, significantly increased the retargeting index of adenovirus containing cyclic but not linear NGR. Since both APN and avb3 integrin are up-regulated in endothelial cells, NGR-bearing adenoviruses could be suitable vectors for tumor gene therapy aimed at inhibition of angiogenesis.

RIBOSOMAL ANTIBIOTICS - BACTERIAL RESISTANCE PROBLEM AND POSSIBLE SOLUTIONS

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The bacterial ribosome is a target for several classes of antibiotics that are currently in clinical use. Among them, two classes are most extensively utilised: possibly the safest macrolide-lincosamide-streptogramin B (MLS) antibiotics and the most potent known aminoglycoside antibiotics. As the worldwide problem of antibiotic resistance is constantly reducing effectiveness of existent drugs, the studies of resistance mechanisms continue to be of the utmost importance.

There are more than a few mechanisms of resistance found for the MLS and aminoglycoside antibiotics. Many of them use strong pumps to expel drugs out of a cell or enzymes that modify either the antibiotic itself or the antibiotic binding site, i.e. RNA. Interestingly, a vast number of clinical strains employ a common mechanism of RNA methylation to protect the protein synthesis machinery from the destructive action of antibiotics. This is a particularly powerful system horizontally transferred from many antibiotic producing bacteria.

In contrast to other target site modifications, like RNA mutations that are found only in a portion of the rRNA genes, the methylation of the antibiotic target is an extremely efficient mechanism, since it modifies all rRNA copies and generates high levels of resistance. The most prevalent mechanism of the MLS resistance is exhibited by the action of the enzymes from the erythromycin ribosome methylase (Erm) family. Erm enzymes methylate a specific adenine residue within 23S rRNA, thus preventing antibiotic binding to the ribosome.

On the other hand, the self-defense mechanism of natural producers of deoxystreptamine-containing aminoglycoside antibiotics is methylation of a specific guanine residue within the 16S rRNA. In addition, this is a relatively new mechanism of resistance found in members of the clinically important *Enterobacteriaceae* family and in the *Pseudomonas aeruginosa* and *Serratia marcescens* clinical strains. Methylation of 16S rRNA in aminoglycoside resistant bacteria is carried out by enzymes from the aminoglycoside resistance methylase (Arm) family.

The results of recent biochemical studies on members of both Erm and Arm family are presented and discussed in light of possible application in design of specific methyltransferase inhibitors. Potential solution will be put in perspective to propose new directions to overcome the threatening problem of MLS and aminoglycoside resistance.

NUCLEIC ACIDS QUANTIFICATION BASED MOLECULAR ASSAY OF THE SURVIVAL OF BACTERIA DURING MINIMAL PROCESSING IN BEEF MODEL

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Sublethally damaged microbes form a potential risk in food stability and safety. Optimisation of process protocols can be based on a reliable detection technique for the fate of food microbe populations. The growth of surviving spoilage-causing and pathogenic bacteria in a beef model system was therefore investigated in parallel with photometric growth and activity measurement and with quantification of 16S r nucleic acids by real-time PCR technique. Minced beef was separately inoculated with *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Listeria monocytogenes* (OKI 9164), *Enterococcus faecalis* (ATCC 29212), *Salmonella Derby* (ELTE 8298), *Deinobacter* sp. (ELTE B126) to get 10^9 g-1 cell count. Preservation treatments were high hydrostatic pressure (100-600 MPa for 1-4 x 5 min) and gamma irradiation (1-4 kGy). Samples were investigated directly after treatment, and following an 8 day storage at 10°C. Tenfold dilution series were made in glucose and peptone supplemented Bushnell Haas broth and distributed into 96 well microplates for the determination of growth and activity curves taken at two different wavelengths (492 nm, cell density; 620 nm resazurine reduction capacity) for 96 hr at 30°C incubation. DNA and RNA were isolated from adequate parallel dilutions, and 16S r nucleic acids were quantified in real-time PCR using universal primers and SYBR green staining. The experimental data were evaluated with mathematical-statistical methods.
It could be observed that growth and biochemical activity curves showed a parallel pattern. It seems to be evident at pressure treatments that the lag period of growth increases as a function of the treatment dose (pressure values/repetitions), proportionally to the pressure tolerance of the test microbes. A similar pattern could also be found at maximum cell yield values. At gamma irradiation a long lag period could be observed, while growth was severely retarded. However, slow but continuous changes in the activity curves referred to metabolism (possibly repair) processes. At microorganisms with high rrn copy numbers (e.g. *Salmonella Derby*), nucleic acid-based quantifications proved the results of photometric growth and activity based investigations. Sublethal damage with active repair processes could be interpreted when high rRNA content was accompanied with low rDNA values. At microbes with low rrn copy number (e.g. *Deinobacter* sp.) well regenerated sublethal damage seems to be atypical.

CHANGE OF ACID PHOSPHATASE ACTIVITY IN A HEAVY METAL POLLUTED SOIL

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Phosphorus is an essential element for all living organisms, playing role in many metabolic processes, primarily in energy transformation. Soils are known to vary widely in their P supplying capaci-

ties, because only a small fraction of the total phosphorus is in available form. Phosphatases have a key role in the cycling of phosphorus. Soil microbial community and plants roots are the major sources of soil phosphatases, which can persist on the surface of clay or humus colloids of the soil for a long time. Their activity is affected by numerous ecological factors: soil organic carbon, phosphorus, clay and lime content and soil pollution.
In the present study, acid phosphatase activity of the rhizosphere of winter rape (*Brassica napus* L.) was investigated in a heavy metal polluted soil. Source of heavy metal pollution was the well known Pb/Zn mine at Gyöngyösoroszi (North-Eastern-Hungary). A significant increase in toxic (As, Cd, Pb, Zn) and nutrient elements (P, Ca, S) was determined in the soil due to the pollution. Activity of acid phosphatase, similarly to the results of willow rhizosphere (in press), increased in the polluted soil. Phosphatase production of living organisms may have been further increased by the lower available P content of the polluted soil - because LE-soluble phosphorus can be easily bound by the higher Ca, Zn, Cd and Pb content in the form of calcium-phosphate and heavy metal salts - and stimulated by the higher water content of the flooding zone of the Toka-creek.

FIRST DETECTION OF HUMAN ASTROVIRUSES IN RAW SEWAGE SAMPLES IN BARANYA COUNTY, HUNGARY

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Routine procedures for monitoring viruses in water samples are not part of the water-microbiology screening panel. Enteric viruses, including astroviruses, are able to persist under environmental conditions and may cause public health problems by contaminating natural and drinking water resources. The aim of the study was to detect human astroviruses (HAstVs) from raw wastewater samples.
To get data on whether HAstVs are spread in the community, 35 raw sewage samples from 22 sewage plants in different regions of Baranya County, Hungary were tested for astrovirus using the polyethylene-glycol method for concentration and the guanidinium thiocyanate-silica procedure for extraction of viral RNA. Reverse transcription-polymerase chain reaction with HAstV-specific primer pair Mon2/PRBEG and Mon2/JWT4 was used for amplification, and the specificity of amplicons was confirmed by sequence analysis. Of the 35 raw sewage samples, 15 (43%) contained HAstV and by sequence analysis, 11 genotype HAstV-1 and 1 genotype HAstV-2 were identified. Three samples were untypeable. This investigation applied for the first time a molecular virological method and the viral screening test in conjunction with a routine bacterial and chemical water assay in order to detect human astroviruses in sewage in Hungary.

THE ANALYSIS OF ARCHAEA COMMUNITY IN GROUNDWATER CONTAMINATED BY CHLORINATED ALIPHATIC COMPOUNDS

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Halogenated hydrocarbons are common contaminants in soil and groundwater in Hungary. Because of their acute and chronic toxicity, the presence of organohalides in the environment is of major concern and a threat to human and environmental health. These compounds are recalcitrant to aerobic dehalogenation because of their physicochemical properties, and have the tendency to accumulate in anoxic ecosystems, including sediments, harbor sludge, soils and groundwater. A potential solution of biological degradation is the use of microbial metabolism for in-situ biodegradation.
For enhanced biodegradation, some substrates can be used that stimulate microbial consortia active in degradation. Methanol (METH), molasses (MOLA) and whey (WHEY) was used as electron donors. Three wells were analysed, one with high (KEW 7) and two with low sulfate (JEW 18 and JW 18/2) concentration. Chemical analysis was performed according to standards; the following parameters were examined: pH and concentration of SO_4^{2-}, Cl^- and halogenated hydrocarbons. Genomic DNA was isolated from groundwater and a section of the 16S rDNA was amplified with Archea specific primers. The diversity of the PCR products was analyzed by DGGE. The DGGE patterns were examined by the Phoretix software and a dendrogram was created. Bands were cut out from the gel for identification by sequence-analysis. It could be concluded that samples from well KEW 7 and well JW 18/2 separated into two clusters, but the samples from JEW 18 did not. Wells KEW 7 and JW 18/2 treated with molasses differed from the control samples, but samples treated with methanol did not, possibly because these samples had a higher redox potential. Samples from well JEW 18 were filtrated and DNA was extracted in low concentration, thus the pattern of samples was not typical and there was no definite group-formation. No treatment microcosms of JW 18/2 and JEW 18 created closely related groups. Microcosms of JEW 18 METH and MOLA grouped together, but separated from the other samples. The microcosm experiment was set up to model biodegradation of trichloroethylene. Whey, methanol, and molasses were added to the bottles, as supplementary substrates. Both whey and molasses were found to enhance degradation. In the case of archaeal DGGE pattern analysis, the molasses and lactate-amended microcosms were similar but separated from the controls.
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GENETIC VARIABILITY OF *GAG* AND *ENV* REGIONS OF HIV-1 STRAINS CIRCULATING IN SLOVENIA

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HIV strains display an extraordinary genetic diversity. They have been classified into three groups: M (main), N (new or non-M, non-O) and O (outlier), which are highly divergent and thought to have arisen from separate zoonotic transmissions. The viruses responsible for the global HIV epidemic fall primarily within group M, which has been subdivided into nine subtypes: A, B, C, D, F, G, H, J and K. Subtypes are equidistant from one another, with inter-subtype diversity ranging from 15% to 22% in the *gag* gene and from 20% to 30% in the *env* gene. Intra-subtype diversity ranges from 3% to 10% in the *gag* gene and from 5% to 12% in the *env* gene. There are sequences that fall between the

intersubtype and intrasubtype distances, and have been designated as sub-subtypes: F1, F2, A1, and A2.
The mechanisms for the generation of HIV variation include an error-prone reverse transcription and recombination. The latter can occur within individuals who become infected with multiple subtypes, resulting in recombinant virus strains with mosaic genomes. To date, 16 recombinants spread widely through populations and are referred to as circulating recombinant forms (CRFs). The greatest genetic diversity of HIV-1 is found in sub-Saharan Africa. All of the known subtypes and many of the CRFs were discovered there. Globally the predominant viral forms are subtypes A and C followed by subtype B and recombinants CRF01_AE and CRF02_AG. Subtype B is the predominant at present in Central and Eastern Europe, but an increase in non-B HIV-1 infections has been observed recently.
To gain insight into the genetic diversity of HIV-1 viruses associated with the HIV-1 epidemic in Slovenia, 20 specimens from HIV-1 infected individuals were classified into subtypes by the sequence-based phylogenetic analysis of the *gag* (p24) and *env* (C2V3) regions of viral genome. The phylogenetic tree based on *env* C2V3 sequences showed that 15 out of 20 samples were subtype B, two A1, one F1, one CRF01_AE and one CRF02_AG. The phylogenetic analysis of the *gag* gene yielded similar results, but one sample had a discordant subtype, since it was identified as subtype A1 in the *env* and AE in the *gag* region. We concluded that subtype B predominates in Slovenia, but non-B subtypes are also present at low percentage, most of them being infected abroad.

BACTERIAL SINGLE-STRANDED DNA-BINDING PROTEINS ARE PHOSPHORYLATED ON TYROSINE

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Single stranded DNA binding proteins (SSBs) are ubiquitous proteins that bind DNA in a sequence-independent manner to maintain genome integrity at various stages of DNA metabolism such as replication, recombination and repair. Besides stabilizing single-stranded DNA (ssDNA), SSBs interact with enzymes such as DNA polymerase, RNA polymerase or DNA helicase, thus modulating their activity. Although accomplishing similar functions, bacterial and eukaryotic SSBs differ considerably in their structure. It is known that eukaryotic SSBs are regulated by phosphorylation on several serine and threonine residues, however, to our knowledge, phosphorylation of SSBs in bacteria has not been yet reported. A systematic search for phosphotyrosine-containing proteins in *Streptomyces griseus* by immunoaffinity chromatography identified bacterial SSBs as a novel target of bacterial tyrosine kinases. Since genes encoding protein-tyrosine kinases have not been recognized in streptomycetes, and SSBs from *Streptomyces coelicolor* and *Bacillus subtilis* share 40% identity, we used a *B. subtilis* protein-tyrosine kinase YwqD to phosphorylate two cognate SSBs (Ssb and YwpH) *in vitro*. We demonstrate that *in vivo* phosphorylation of *B. subtilis* Ssb occurs on tyrosine residue, and that this reaction is affected antagonistically by kinase YwqD and phosphatase YwqE. Phosphorylation of *B. subtilis* Ssb increased binding to single-stranded DNA *in vitro*. Tyrosine phosphorylation of *B. subtilis*, *S. coelicolor*, and *Escherichia coli* SSBs occured while they were expressed in *E. coli*, indicating that tyrosine phosphorylation of SSBs is a conserved process of post-translational modification in taxonomically distant bacteria.

CONTRIBUTION OF QUORUM SENSING MOLECULES TO THE VIRULENCE OF *PSEUDOMONAS AERUGINOSA* IN AN EXPERIMENTAL URINARY TRACT INFECTION MODEL

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Quorum sensing signals were proposed to play the most important role in the pathogenesis of respiratory tract and burn wound infections caused by *Pseudomonas aeruginosa*. This pathogen was reported to monitor its cell density as well as expression of virulence determinants by quorum sensing signal mechanisms operating through auto-inducers. In the present investigation, we studied the contribution of quorum sensing signals during the course of *P. aeruginosa* induced urinary tract infection (UTI). Fifty uroisolates and one standard strain of *P. aeruginosa* were initially screened for the production of quorum sensing signals both qualitatively and quantitatively. For further studies, quorum sensing positive and quorum sensing negative strains (2) of *P. aeruginosa* were checked for their virulence in an acute ascending UTI mouse model. It was observed, that the quorum deficient strain was significantly less virulent than the quorum sensing producer strain during the course of infection. The present study points out that quorum sensing signals are important for the pathogenesis of urinary tract infections caused by *P. aeruginosa*.

CONTRIBUTION OF MACROPHAGE SECRETORY PRODUCTS TO VIRULENCE OF *PSEUDOMONAS AERUGINOSA* IN URINARY TRACT INFECTION

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The primary line of innate defense against most bacterial pathogens consists of resident macrophages residing in tissues and polymorphonuclear neutrophils (PMNs) migrating from the blood to the site of infection. Following stimulation of macrophages, secretory products are produced. The large array of biomolecules present in macrophage secretory products (MSPs), including pro as well as anti-inflammatory cytokines, can influence the ultimate outcome of an infection. The present investigation was planned to study the contribution of MSPs produced in response to interaction of macrophages with *P. aeruginosa* to virulence of this pathogen in a murine model of ascending urinary tract infection. Organisms grown in the presence of MSPs were more virulent as indicated by a significant increase in renal bacterial load, tissue pathology, malionaldehyde production and neutrophil recruitment. The results of the present study points out that pathogens possess mechanisms to exploit host defense mechanisms for their own survival leading to chronicity and recurrence of infections. Implications of these findings in relation to UTIs caused by *P. aeruginosa* are discussed.

INVOLVEMENT OF G PROTEIN MEDIATED SIGNALLING PATHWAYS IN THE REGULATION OF AUTOLYSIS IN *ASPERGILLUS NIDULANS*

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G-protein-coupled receptors have a central role in sensing various environmental changes and signals such as depletion of nutrients, oxidative stress, or presence of hormones and pheromones. In the *Aspergillus nidulans* genome nine putative GPCR coding genes (gprA–L) were identified. The suitable G protein pairs of the GPCRs have not been found yet, despite the fact that there are three known G protein α subunits (FadA, GanA-B) in *Aspergillus nidulans.* Heterotrimeric G proteins are controlled by RGS (regulator of G protein signalling) proteins of which four (flbA, rgsA-C) have been found by this time. These proteins increase the GTPase activity of Gα subunits, and therefore inactivate them. The RGS pair of FadA is FlbA, while GanB is controlled by RgsA. The FadA/FlbA pathway maintains vegetative growth, represses asexual sporulation and sterigmatocystin production ,while GanA blocks conidiogenesis and induces germination of conidia. The GanB/RgsA pathway represses asexual sporulation, sterigmatocystin synthesis and vegetative growth, stimulates the germination of spores, and also induces oxidative and heat stress tolerance. The aim of our study was to investigate the role of fadA/flbA and ganB/rgsA gene pairs in the regulation of autolysis of *Aspergillus nidulans* using the fadAG203R, ΔflbA, ΔganB, ΔrgsA and ΔrgsAΔganB mutants.

Our results indicated that both pathways had an important role in the regulation of extracellular proteinase and γ-glutamyl transpeptidase production during autolysis and, therefore, they were crucial in the mobilisation of organic nitrogen sources (e.g. proteins and glutathione) in carbon starving cultures. Deletion of ganB or rgsA genes reduced the activity of both proteinase and γ-glutamyl transpeptidase, while the mutations in the FadA/FlbA pathway caused only a time shift in the induction of these enzymes. The FadA/FlbA pathway also affected the fragmentation of hyphae, but neither the FadA/FlbA pathway nor the GanB/RgsA pathway significantly influenced the extracellular chitinase activities and the decrease in the dry cell mass during autolysis.

The mutations of the GanB/RgsA pathway also influenced catalase activity. In control cultures, high catalase activity was detected only in the early autolytic phase of growth (24-60 h). In contrast, in the loss-of-function rgsA mutants continuously high catalase activity was measured during both the early and the late autolytic phases of growth (24-168 h). Using RT-PCR, we found that the altered regulation of catB could be responsible for the high catalase activity in the late autolytic phase of growth in these mutants. Beside of catalase, the FadA/FlbA and the GanB/RgsA pathways did not cause significant changes in the induction of other antioxidant enzymes (e.g. superoxide dismutase, glutathione peroxidase) and did not alter either the accumulation of superoxide and peroxides, or the decrease in the viability of the cultures during autolysis.

PHYSIOLOGICAL ADAPTATION OF *CAMPYLOBACTER JEJUNI* UNDER CONDITIONS OF THERMAL AND OXIDATIVE STRESS

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Bacterial adaptation to environmental stress is recognized as an increasingly important area in microbiology, including food safety concerns, especially in conditions of minimal food processing. Different general and specific stress response mechanisms are known by which bacteria can sense and respond with increased resistance and survival possibilities in the same or other stress challenges.

Although thermotolerant campylobacters are the most commonly reported bacterial causes of acute human enteritis in developed countries, little is known about their ability to cope with hostile conditions in extra-intestinal environments. This is known as »*Campylobacter* paradox«. Campylobacters lack many of the well characterized stress adaptive responses of other food-borne bacteria. However, still unknown mechanisms allow *Campylobacter* cells to adapt and survive at least some kind of

stress, which make them more resistant. Campylobacters may enter into a viable but nonculturable (VBNC) state, where they are still metabolically active and potentially infectious but not detectable on growth media. Therefore, we used alternative methods for the detection of metabolic and virulent properties of stressed cells, based on criteria other than reproduction. Non-starved and starved *C. jejuni* cells from different growth phases after exposure to thermal (4, 25, 48, 55 °C, 3-60 min) or oxidative stress (3 mM H_2O_2, 10 min or atmosphere oxygen incubation up to 72 h) were studied with the use of different indicators: (i) culturability on Karmali agar (ii) viability on membrane integrity changes detected with LIVE/DEAD Bacterial Viability Kit (BacLightTM), SYTO 9, 13 and PI with fluorescent microscope and flow cytometry (iii) morphology changes followed by phase contrast and electron microscopy, (iv) gene expression of selected stress genes by RT-PCR and real-time PCR and (v) virulence on different cell culture models. High temperature and oxidative stress invoked quick transformation from culturable spiral shaped to nonculturable spiral and coccoid cells. Despite many phenotypic physiological changes of cells observed with other methods, we have documented clear difference in the expression of the groEL and rpoD transcripts only after 10 min or longer thermal treatment of cells at 55 °C. Oxidative stress seemed to have a more apprehensive influence on virulence properties of *Campylobacter* cells than thermal stress.
Exposures to atmospheric oxygen concentration and temperature changes are some of the most common stress conditions microaerophilic Campylobacters are exposed to during food processing. Understanding microbial defence mechanisms under stress challenges could be important when designing food safety measures in the food chain. In addition, the pathogen adaptive stress response could be crucial for the outcome of host-pathogen interaction.

NO EVOLUTION OF VIRULENCE IN THE SWISS HIV-1 EPIDEMIC

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Being the most recent addition to the list of major human pathogens, human immunodeficiency virus type 1 (HIV-1) may still be evolving to adapt to its new host species. Whether the virulence of the virus, i.e. its ability to cause disease, is evolving towards higher or lower levels, is highly relevant for the future of the AIDS pandemic. We analyzed the correlation of various markers of disease progression with the date of infection in antiretroviral naïve patients in the Swiss HIV Cohort Study (SHCS) over 20 years. We found that the virulence of newly diagnosed HIV-1 infections has fluctuated around a stable level during the two decades of the epidemic, which indicates the absence of directional evolution of virulence. The results suggest that the virus may have already evolved to optimal virulence in the human host, or the evolution of virulence may be subject to evolutionary constraints.

ROLE OF E-CADHERIN TUMOR SUPPRESSOR GENE POLYMORPHISM IN THE HUMAN PAPILLOMAVIRUS (HPV) ASSOCIATED MALIGNANCIES

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The genital tract is the main reservoir of mucosal human papillomaviruses (HPVs) and is home to more than 25 HPVs. A large majority of HPVs are found in the lesions spanning the entire spectrum of cytological abnormalities, from low-grade SIL to invasive cancer. High risk HPV16 and HPV18 are frequently detected in malignant tumors and are associated with the majority of cervical carcinomas, which are one of the most common female malignancies worldwide.
The larynx is another significant anatomic site in terms of HPV involvement, exceeded in clinical importance perhaps only by the genital tract and skin infections. This is because HPV infection is the etiological agent of a clinically significant disease known as laryngeal papilloma (papillomatosis).
E-cadherin plays a critical role in epithelial cell-cell adhesion and the maintenance of tissue architecture. Perturbation of E-cadherin mediated cell adhesion is involved in tumor progression and metastasis. In vitro loss of E-cadherin expression is associated with the loss of cellular differentiation and increased cellular invasiveness and infiltration.
Single nucleotide polymorphisms are common DNA sequence variations among individuals, which may significantly advance our ability to understand and treat human disease, including cancer. SNPs, particularly in gene promoters, may be important to gene functions and/or transcriptional efficiency. Two functional promoter polymorphisms have been identified within the E-cadherin promoter, at nucleotides -347 G→GA and -160 C→A relative to the transcriptional start site. First, the E-cadherin -160 C→A polymorphism was shown to suppress E-cadherin expression, and has been associated with gastric, breast, colon, bladder, and prostate cancers. A second association has recently been shown between the -347 G→GA E-cadherin polymorphism and familial gastric cancer, indicating that single nucleotide polymorphisms within the E-cadherin promoter may be associated with cancers.
In the present study, we investigated nucleotide variations in the promoter region of the E-cadherin gene (CDH1). We examined the allele frequencies of SNPs in the CDH1 promoter in a control population and Hungarian patients with cervical carcinoma or head and neck cancer. The relations between HPV infection, metastasis formation and the sequential alterations of E-cadherin promoter are also being studied.

MULTIFUNCTION ION AIR CLEANING TECHNOLOGY IN MICROORGANISM-DECONTAMINATION

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In many branches of medicine, microbiologically clean air is a significant condition either of microbial pollution or infection reduction, i.e. in operating room, or medical staff infections.
The MFI method (Multifunction Ion Air Cleaning) of Genano Oy, Finland was examined, using Nanobio E310 device: www.wpip.pl, with regards to its air decontamination efficiency. In this study,

two co-operating processes are used: ionization and electrostatic attraction of particles and microorganisms from polluted air. The work parameters of the tested devices are: cleaning capacity 250 m^3/h, at air flow velocity 0,5 m/s. The microbiological purity of the exhaust air was measured. Two methods were applied for qualitative and quantitative tests; De Ville Biotechnology (MicroBio device), and contact plates method (Oxoid). There were 10 test bacterial and fungal types from ATCC collection (Rockville), preserved in MicroBank PRO-LAB Diagnostic sets, in -70°C temperature. For quantitative test, a microbial suspension (aerosol), density 10^6 – 10^8 cfu/ml was used under aseptic conditions. Microbiological purity of air, after passing through the air cleaning MFI device, ensured the removal and destruction of both bacteria and fungi. It is to be highly effective towards a wide spectrum of microbes: bacteria including *Bacillus subtilis* spores, fungi i.e. *Aspergillus niger*, however, the highest effectiveness occurs in attitude to Gram-positive i.e. *Staphylococcus aureus* and Gram-negative rods *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens*. The extent of microbiological air contamination does not influence effectiveness of air cleaning with the MFI method. The long-term (7 days) work of Nanobio E310 device does not result in any change in the efficiency of air cleaning with the MFI method, even under conditions of high microbiological pollution of the internal air. Experiments point out a significant reduction of microbes in cleaned air. The end result shows at least 10^8 less bacteria and fungi than in the beginning of test, which equals to a 99,999% effectiveness at a particle range of ≥ 0,003 µm. Microbiological air purity passing the MFI air cleaning technology process (<1 cfu/m^3) correponds to the highest air cleanness class.
The application of the MFI technology, assuming appropriate assembling of air cleaning devices and preserving the air exchange frequency adequate to room cubature, equipment and number of employees, enables reaching at least class B microbial air purity: 100, M3.5 air class, ISO 5 acc. to U. S. Federal Standard 09E and ISO standards as well.

APPLICATION OF IMAGE ANALYSIS OF FUNGAL COLONIES FOR ASSESSMENT OF EFFECT OF HEAVY METALS

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Because of various effects of heavy metals on the growth and branching of hyphae, the measurement of colony extension may lead to misleading conclusions. We developed a simple method of in depth analysis of mycelial morphology for more appropriate assessment of changes in colony structure and development rate. Strains of thirteen *Trichoderma* species were used to verify that the colonies grown on thin agar film produce a quasi two-dimensional picture when they are digitalized with an official scanner and analyzed with ImageJ software. Twenty-two strains of 13 *Trichoderma* species (*T. asperellum*, *T. atroviride*, *T. aureoviride*, *T. ghanense*, *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. parceramosum*, *T. piluliferum*, *T. pseudokoningii*, *T. reesei*, *T. saturnisporum*, and *T. virens*) from artificially polluted or non-polluted areas were used to assess the effect of heavy metals on growth and colony morphology. Concentration series (0.1-10.0 mM) of Cd, Co, Cu, Hg, Mn, Ni, Pb, Zn prepared in medium-strength PDA medium were tested. We found that the particular heavy metals influenced the mycelial development in various manners, although all of them caused a significant reduction of colony growth. Cd and Hg consistently reduced the fractal dimension (D-value) of colony border suggesting that the branching system was strongly disturbed. Increased D-value was observed at colonies grown on Co, Ni, and Pb polluted media, whereas the effect of Cu and Mn varied by strains. Zn was the only metal the ions of which did not cause significant colony alterations at the tested range. Nine strains of *T. virens* originating from artificially polluted soils reacted in the same direction to the treatments, irrespective of the particular heavy metal content of the hosting soil.

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THE INCIDENCE OF *MYCOPLASMA BOVIS* MASTITIS IN CROATIA

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A significant increase in severe clinical mastitis cases resistant to antibiotic therapy was observed on a large farm with approximately 1800 dairy cows at different stages of lactation. The animals were of Frisian Holstein breed, purchased from abroad two or three years ago. Acute forms of mastitis were characterised with rapid decrease in milk production, and quick spreading of infection from affected quarters to others. Abnormal udder secretion varied from watery milk to a colostrum-like fluid or purulent material. In some cases, shortly after milking an abuundant flaky or sandy, granular sediment settled down in the tube with a clear liquid above it. As mycoplasmal mastitis was not identified yet in Croatia, the animals were unsuccessfully treated with different antibiotics for several months. Finally, randomly taken udder secretions of 50 cows were cultured on bacteriological media and mycoplasma liquid and solid media. Streptococci of Lancefield B or D groups were isolated from the udder of 10 animals. Other samples were bacteriologically negative, but some of them contained antibiotics. Using Sandwich ELISA mycoplasma isolates from the udder of 15 animals were identified as *Mycoplasma bovis*.

THE DEEP-ROUGH LPS PHENOTYPE EXHIBITED BY RFAH MUTANTS OF *SALMONELLA ENTERICA* RESULTS IN DOWNREGULATION OF SPI-1 AND SPI-4 GENES

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The lack of transcriptional regulator RfaH has been reported to attenuate virulence of *Salmonella enterica* sv. Typhimurium strain SL1344. Oral vaccination using the attenuated mutant elicits a protective immune response against subsequent challenges with the isogenic wild-type strain.
We show that *rfaH* mutants are able to invade eukaryotic cell lines more efficiently than wild-type parental strains. Intracellular growth of the mutants, on the other hand, is severely impaired in both epithelial and macrophage cells. The decreased intracellular growth is a consequence of higher vulnerability of the mutants, rather than a slower replication at the intracellular milieu. In vitro tests suggest that the higher intracellular death rate of *rfaH* mutants may originate from an increased susceptibility to small intracellular cationic peptides.
In order to determine the genes responsible for the virulence attenuation and/or decreased intracellular growth ability, microarray experiments were performed to compare transcriptomes of different prototype *S. enterica* strains with those of their isogenic *rfaH* mutants. RfaH was proven to be directly involved in the expression of the *waa* and *wbb* operons encoding the LPS core and O-antigen,

respectively. Additionally, expression of *siiABCD,* located on the *Salmonella* pathogenicity island-4 (SPI-4), was found to be downregulated in *rfaH* mutants, suggesting that these genes form one single transcriptional unit. Since this operon carries the short upstream *cis*-acting sequence required for the RfaH-dependent regulation, transcription of *siiABCD* appears to be directly influenced by RfaH. On the other hand, several additional genes involved in sugar metabolism, membrane composition, type-three secretion system-1 (TTSS-1, encoded on SPI-1), etc. are indirectly downregulated in *rfaH* mutants due to their deep rough LPS phenotype. Deletion mutants lacking the SPI-4 genes exhibit high virulence attenuation in a murine model of salmonellosis. Furthermore, TTSS-1 was also shown to be required for the virulence of *Salmonella*. Our results suggest that virulence attenuation of LPS deep-rough mutants is partially caused by downregulation of virulence determinants encoded on various pathogenicity islands.

MAPPING THE *THERMOPLASMA* PROTEOME - STRUCTURAL PROTEOMICS STUDIES USING FREE-FLOW ELECTROPHORESIS AND CRYO-ELECTRON TOMOGRAPHY

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Thermoplasma acidophilum is a thermoacidophilic archaeon whose genome has been sequenced. To carry out proteomic analysis on this organism, we used cell lysates that were fractionated using free-flow electrophoresis (FFE), a matrix free electrophoretic technique. The FFE separates the cytoplasmic proteins into about 70 fractions according to their isoelectric point. Afterwards, single fractions were studied by means of cryo-electron tomography (cryo-ET), which allowed obtaining three-dimensional (3-D) structural information on pleiomorphic frozen-hydrated biological specimens, preserved in their native state by vitrification. It is well suited for analysing structures ranging from separated macromolecules to cells *in toto*.
We used cryo-ET for the 3-D structural characterisation of isolated complexes at a resolution of 2-3 nm. Additionally, the contents of single fractions were characterised by polyacrylamide gel electrophoresis and mass spectrometry. The knowledge of the 3-D structure and the determination of the identity of different proteins will enable us to generate a template library of protein complexes that is going to be used as an input for pattern recognition algorithms designed to search electron tomograms of intact ice-embedded cells. The final aim of these studies was to locate and quantify the different macromolecular assemblies within a 3-D reconstruction of a whole *T. acidophilum* cell. This will lead to a protein map of the intact cell and reveal the intracellular molecular organisation of the complexes in their natural environment.

THE CYTOSOLIC AND MACROMOLECULAR SUBPROTEOME OF *THERMOPLASMA ACIDOPHILUM*

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A semi-automatic, high-throughput procedure for protein identification, comprising 2-dimensional gel electrophoresis coupled with MALDI-TOF mass spectrometry, was used to obtain a global view concerning the cytoplasmic proteins expressed by *Thermoplasma acidophilum*. In addition, glycerol gradient ultracentrifugation was used to identify subunits of several macromolecular complexes. With the 2D electrophoresis proteome approach, over 900 spots were resolved from which 272 proteins or protein-containing complexes were identified. These included a significant number that form macromolecular complexes and were determined to be expressed at high levels, among them ribosome, proteasome, thermosome, VCP-like ATPase, succinyl CoA synthase, ornithine carbamoyl transferase, glutamine synthase, glutamine hydratase, peroxiredoxin. We found 15 proteins in the heavy glycerol-gradient fractions that were putative building blocks of protein complexes. The function and structure of several of these proteins and complexes, like that of the ribosome, translation initiation factor eIF-6 related protein, elongation factor 1, DNA-dependent RNA polymerase and pyruvate dehydrogenase, are well-studied in other organisms, whilst others (Ta0078, Ta0316, Ta0341, Ta0522, Ta0890, Ta1155, Ta1201) are less well-known. The majority of these proteins belong to the categories of hypothetical or conserved hypothetical proteins, and they are present in the cytosol at low concentrations. Although these proteins exhibit some homology to known sequences, their structures, subunit compositions and biological functions are not yet known.

HTLV: MORE THAN THE FIRST HUMAN RETROVIRUS

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Human T-cell leukemia/lymphoma viruses, HTLV-1/2 were described in 1980 as the first human retroviruses. Very recently, HTLV-3 and HTLV-4 have been isolated in Cameroon and Gabon indicating present day jumps of counterpart primate STLVs as zoonosis to humans. HTLV-1 has been etiologically associated with adult T-cell lymphoma/leukemia (ATL), tropical spastic paraparesis (TSP), dermatomyositis and infective dermatitis (ID) with abnormalities of immune function. The role of HTLV-1 in the development and progression of cutaneous T-cell lymphoma, mycosis fungoides and Sezary sy. has not yet been clearly established. Central to the tumor-inducing activity of this virus is the Tax protein, which transactivates a number of adjacent or distant cellular genes as well as regulatory boxes in the proviral LTR.

Accumulated evidence demonstrate that exogenous infection by human retroviruses induces local and/or systemic eosinophilia with various forms of immunological disorders as a consequence of the expression of viral genetic elements directly or as a promoter of some cytokines. Here we report the possibility of HTLV infection in our patients with various dermatoses and eosinophilia.

440 patients with cell-proliferative diseases and/or dermatoses and healthy donors were screened for serum antibody to HTLV by IIF and ELISA. DNA samples of skin lesions and lymphocytes from 50 patients were analyzed by PCR amplifying a 158 bp segment of 3' tax and a 210 bp (1323-1442) conserved fragment of gag of HTLV-1. Amplified PCR products were inserted into a pGEM vector and cloned in *E.coli* XLI-Blue strain. GM-CSF, IL-3,IL-5 and Eotaxin were quantitatively determined in patient sera by the respective ELISAs. T cell receptor gene rearrangement was analyzed by Vγ and Jγ primers. Based on serology, no confirmed *exogenous* infection was found. Molecular detection by PCR however indicated the presence of HTLV-related DNA sequences in patients with dermatoses and eosinophilia. HTLV-related DNA sequences were detected more frequently in lymphoreticular proliferations (62%) than in bullous dermatoses (17%) or inflammatory diseases (16%). DNA hybridization data as well as nucleotide sequence analysis identified retroviral elements homologous (>95%) to HTLV-1 *gag*. No homology higher than 5% to related sequences including several known

endogenous human retroviruses was found. Among the *eosinophil chemotactic factors,* GM-CSF was increased in cultured lymphocytes stimulated by IL-2 in 40% of all patients studied. In the serum elevated levels of IL-5 and Eotaxin could be detected in 50% and 32% of all patients respectively. No increase of IL-3 was observed however.

It is assumed that in Hungary, where direct evidence of exogenous HTLV infection has not yet been confirmed, restricted expression of HTLV-related retroviral sequences or defective HTLV-1 proviruses with internal deletions are involved in the progression of diseases with altered immunological responsiveness. Moreover, HTLV-1 *tax* could transactivate gene(s) of various eosinophil chemotactic factors (GM-CSF, IL-5, Eotaxin), which results in reactive local and/or systemic eosinophilia.
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COMPARATIVE STUDY OF TOXIC HEAVY METAL ION TOLERANCE AND ACCUMULATION BY FUNGI

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The tolerance and accumulation of toxic heavy metal ions (As^{5+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, $Cr_2O_7^{6-}$, Cu^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}) by fungi (*Alternaria sp. (2), Aspergillus amstelodami, A. niger (3), A. parasiticus, A. tamarii, Cladosporium chlorocephalum, C., Fusarium sp., Geotrichum candidum, Penicillium chrysogenum, P. corylophilum, P. ovetense, P. purpurogenum, Rhodotorula glutinis, R. rubra, Saccharomyces cerevisiae*) were studied in serial experiments.

Fungal strains were isolated from the laboratory air on Sabouraud's agar medium containing ten heavy metal ions [Na_2HAsO_4, $BaCI_2$, $Cd(NO_3)_2$, $CoCI_2$, Na_2CrO_4, $CuSO_4$, $HgCI_2$, $MnCI_2$, $NiCI_2$, $Pb(NO_3)_2$] each in 1 mM concentration. Growth of the previously isolated strains were tested for 10 mM (0.5-2 g/l) concentration of ten heavy metal ions on the Sabouraud's agar medium to select more tolerant fungal strains. The metal ion uptake by shaken resting or proliferating cells of the tested fungal strains was determined with atomic absorption spectrophotometry of the unabsorbed (remaining) amount in the supernatants or by radioactive labelling for kinetics studies with yeasts by measuring the radioactivity of filtered cells. Some fungi being exposed to toxic heavy metals were able to tolerate much higher concentrations of these ions than usual. The kinetics of accumulation was tested by radioactive labelling. These preliminary studies have shown the possibilities of the various types of uptake kinetics (e.g. active, passive transport).

By cross testing tolerance at the 10 mM level, many multitolerant strains could be established - such as *Aspergillus niger* #21 (for As, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb); *Rhodotorula glutinis* (for As, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb); *Aspergillus tamarii* (for Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb); *Cladosporium oxysporum* (for As, Ba, Cd, Co, Cr, Cu, Mn); *Penicillium chrysogenum* (for As, Ba, Co, Cd, Mn, Ni, Pb); *Alternaria sp.* #1 (for As, Ba, Cd, Co, Cu, Mn); *Alternaria sp.* #47 (for As, Ba, Cr, Cu, Mn, Pb); *Aspergillus parasiticus* (for Ba, Co, Cu, Mn, Ni, Pb); *Penicillium purporogenum* (for As, Ba, Cr, Cu, Mn, Pb); *Penicillium ovetense* (for As, Ba, Co, Cr, Mn, Ni); *Aspergillus niger* #37 (for Ba, Co, Cu, Mn, Ni2); *Aspergillus amstelodami* (for As, Ba, Co, Cu, Mn); *Cladosporium chlorocephalum* (for As, Ba, Cr, Cu, Mn); *Penicillium corylophilum* (for Ba, Co, Mn, Ni, Pb); *Rhodotorula rubra* (for As, Ba, Mn, Pb); *Saccharomyces cerevisiae* (for Ba, Cr, Mn, Ni); *Aspergillus niger* #12 (for Cu, Mn, Ni); *Geotrichum candidum* (for Ba, Mn, Pb) and *Alternaria sp.* #52 (for Ba, Cu).

The elimination of heavy metal pollutants cannot be solved solely by industrial absorbents but also by natural biological ones - like fungi - that are important for the health of mankind. The possible relations of the tolerance and uptake of heavy metal ions by fungi was examined – the atoms separately

treated as they are in the periodic table according to the group number (e.g. uptake between Ba & Cd [r=0.759**, DF:11]); tolerance *Alternaria* spp. & *Aspergillus* spp. vs. group number 2 & 8)

INCREASED EXTRACELLULAR CHITINASE PRODUCTION IN SOLID SUBSTRATE FERMENTATION BY *TRICHODERMA HARZIANUM* HAPLOTYPE AA

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Chitin is composed of ß(1-4) linked units of the amino sugar N-acetyl-D-glucosamine, and it is the second most abundant renewable resource after cellulose. Chitin and its derivatives are used for a number of commercial products such as medical applications (i.e. surgical thread), cosmetics, dietary supplements, agriculture and water treatment, and hydrolysis to N-acetyl-glucosamine, which is required for pharmaceutical applications. Species of *Trichoderma* have frequently been reported to be good sources for chitinolytic enzymes, but no high-yielding fermentation processes have as yet been described. In addition, the identification of producer strains for the industry is still empiric, supported by high-throughput methods. On the other hand, there is accumulating evidence that the genetic diversity of filamentous fungi correlates with phenotypic (metabolic) diversity. To put this to a test, we have investigated a pool of strains of *T. harzianum* for their ability to produce chitinases in solid state fermentation. Extracellular chitinase production of *Trichoderma harzianum* isolates belonging to these four haplotypes were investigated in solid substrate fermentation (SSF) using wheat bran and crude chitin (SIGMA) in a 9:1 mixture as a substrate and enzyme inducer. Altogether 51 isolates were screened in a 3 and 5 day SSF, namely 16 strains from haplotype Aa, 20 from Ba, 7 from Bb and 8 from C. Strains were isolated from South-East Asia, Siberia, Russia, Africa and South America. We show here that indeed one ITS haplotype of the fungus (Aa) exhibits statistically supported superior chitinase production over the other haplotypes.

NEW POSSIBILITIES FOR THE PRODUCTION OF THE GLYCEROL DERIVATIVES WITH ENZYMATIC BIOCONVERSION

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Glycerol is a by-product of biodiesel production. The production of such alternative biofuels is increasing rapidly in the countries of the European Union. Glycerol can be utilized in various ways of biological transformation for industrially valuable products. Reuterin (3-HPA), dihydroxyacetone (DHA), and 1,3-propanediol (1,3-PD). 1,3-PD have the largest potential, because it is produced chemically with more then 100.000 t/yr capacity for the polymer industry. Because some bacteria are able to ferment glycerol to 1,3-PD under anaerobic conditions, a new recombinant technology was worked out in the last decade, and a new factory will start with this biotechnological process next year. It will use a recombinant *Escherichia coli*, which first produces glycerol from glycose with the geneproduct enzymes of *Saccharromyces* genus, and with bacterial gen-coded enzymes it can convert glycerol to 1,3-PD under aerobic conditions.

As we reported at the previous Congress of the Hungarian Society for Microbiology, we are working on an alternative new biotechnological way in order to produce 1,3-PD from glycerol using a coenzyme regenerating enzyme membrane reactor. Fermentative production of any molecule always has lower yields than an enzymatic method, because the cells also incorporate some portion of the raw materials and they need it also for maintenance of their viability. On the other hand, they produce usually a lot of by-products, which can be avoided using enzymatic bioconversion.
For the enzymatic production of 1,3-PD from glycerol, two key enzymes are needed: Glycerol-dehydratase (GDHt, E.C.4.2.1.30) and a NADH2-dependent 1,3-propanediol-oxydoreductase (PDOR, E.C.1.1.1.202). In our process the NAD+ produced by PDOR can be regenerated by the third key enzyme called glycerol-dehydrogenase (GDH, E.C. 1.1.1.6), producing DHA for the cosmetic industry. Physiologically the glycerol utilization can be done via GDH and the terminal oxidation, but under anaerobic conditions the produced NADH2 coenzyme will be regenerated by the PDOR. In our work the enzymes of *Enterobacter aerogenes* are used without any separation and purification to reach a cost-effective technology.
In our previous report we showed, how the three key enzymes can be produced effectively, and also a mathematical model describing the complex enzymatic system producing 1,3-PD and acetic acid (AcOH) as the only by-product.
It is well known from the literature, that because of the suicide inactivation of the GDHt with its co-enzyme (B12), this enzyme must be regenerated by a protein complex which needs ATP for regenerating. But at the same time ATP is the coenzyme responsible for the by-product formation due to the phosphorylation of DHA. Our new mathematical model shows clearly that with this system 1,3-PD and AcOH can only be simultaneously produced, or if we try to inhibit the by-produt formation, the system can not be used for continuous production because of the absence of ATP regenerating steps.

MODELLING OF CONTINUOUS FERMENTATION SYSTEM USING IMMOBILIZED BREWER'S YEAST

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The brewing and distilling industries are economically powerful and thus have always been in the forefront of technological development. The use of an immobilized yeast cell system for alcoholic fermentation is an attractive and rapidly expanding research area, because of the additional technical and economical advantages over the use of traditional batch process with free cells, including rapid processing and high volumetric productivity as well as low capital and production costs. In contrast to the distilling industry, the well-balanced aroma and flavor of the final product in beer production is equally or even more important than the efficient fermentation and high yield. The application of immobilized brewing yeasts for continuous beer fermentation is a challenging opportunity for the brewing industry. Engineering problems linked to the choice of carrier and reactor design are complicated by the effects of immobilization on flavor profile, since beer is a complex aqueous solution containing CO_2, ethanol, inorganic salts and about 800 organic compounds. Indeed, numerous authors have reported differences in specific metabolic activity between free and immobilized yeast on various carriers. Therefore, despite the economic advantages, the continuous process has been so far limitedly applied in the industry, only in beer maturation and alcohol-free beer production. The aim of this study was to evaluate the continuous fermentation system applying immobilized brewer's yeast using SIKUG glass porous carrier.

Some kinetic properties of the applied brewing yeast were determined using media containing glucose or maltose as carbon sources. In the course of propagation on maltose medium, this yeast has a longer lag-phase than on glucose medium, but the specific growth rates were similar in both cases. Maltose medium was selected to propagate cells using a bench top fermentor for immobilization. After harvesting, yeast cells were preserved in a suspension containing 10 % maltose. Carriers were prefilled into a home-made packed-bed bioreactor and sterilized. The immobilization procedure was carried out on a laboratory scale. Cell concentration in the suspension was reduced with about three orders of magnitude. Results showed that most of the cells were bound onto SIKUG glass porous carrier. Different concentrations of maltose substrate were applied to model the continuous fermentation. The flow rate was 195 ml/h and assured by a peristaltic pump.
The results showed that up to 9% maltose the alcohol concentration has grown dynamically. Most of the consumed maltose was converted to ethanol. The bioreactor operated for more than one month with high efficiency. It led to a conclusion that a packed-bed bioreactor using immobilised yeast cells is capable to do continuous primary fermentation process for a long time. More studies are needed such as about using wort, changing the operation parameters and sensory evaluation of the product, to give answers for the question of applying this technology. Undoubtedly, with the use of immobilised yeast cells, the time of the primary fermentation process can be shortened.

INFLUENCE OF DIURNAL REDOX FLUCTUATION ON MICROBIAL ACTIVITY DYNAMICS IN THE RHIZOSPHERE

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Wetland plants release oxygen through the aerenchyma system to the roots providing oxic habitats in the rhizosphere. The consumption of oxygen during the night establishes a diurnal fluctuation of redox conditions. The fluctuating oxic-anoxic environment may explain the coexistence of aerobic and anaerobic microorganisms in the rhizosphere. In order to detect the metabolically active members of the community and to investigate the effect of fluctuation of oxygen concentration on these dominantly active members, RNA-based fingerprinting techniques were applied.
The denaturing gradient gel electrophoresis (DGGE) patterns of 16S rRNA, obtained with universal primers were very similar regardless of the time of sampling, indicating that the overall ribosome level of the predominantly active members did not change significantly. Initial studies showed the daily fluctuation of ammonia concentration in the rhizosphere and that ammonia-oxidizing bacteria (AOB) play a major role in determining nitrification rates in the root environment.
A detailed investigation was carried out on the composition and dynamics of the expressed ammonium monooxygenase genes (*amoA*). It was assumed that the metabolic activity of different nitrifying bacteria might be separated in time. The *amoA* DGGE patterns showed diurnal dynamics with specific bands observed either in day or night samples, while some other *amoA* genes were continuously expressed. This result indicates for the first time, that separation of microbial activities, as a result of fluctuating oxygen availability, can be an important factor of niche differentiation in the rhizosphere.

THE LOCUS CONTROL REGION OF EPSTEIN-BARR VIRUS

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After infection, EBV genomes persist latently in B cells, and other cell types. Both in vitro and in vivo, two major groups of gene expression patterns were observed in latency. The first group, in which the major EBV latency C promoter (Cp) is switched off ('Cp off' latency), excludes the expression of the main immortalizing viral protein EBV nuclear antigen (EBNA) 2 and is found mainly in the primarily malignant tumors: Burkitt´s Lymphoma (BL), Hodgkin's disease (HD) and Nasopharyngeal Carcinoma (NPC) and other epithelial tumors. In 'Cp off' latency, the viral genome maintenance protein EBNA1 is expressed from the Q promoter (Qp). In the second group ('Cp on' latency), which is found mainly in lymphoblastoid cell lines (LCLs) and LCL-like cells in PTLD patients, all EBNAs are expressed from Cp. The highly different gene expression patterns in both latency groups depend on the respective cellular backgrounds and are reflected by widely different chromatin organization patterns of the persisting viral genomes. In 'Cp off' latency, viral episomes are mostly packed as nucleosomes, and their CpG dinucleotides generally show a much higher degree of methylation than in 'Cp on' latency [1,2]. An area of open chromatin within the EBV genome has been mapped to the EBER-oriP locus, which exerts functions of a locus control region (LCR). This viral LCR serves as a transcriptional enhancer for several viral promoters, and regulator for replication and nuclear matrix attachment of the viral genome. The EBER genes code for two small RNAs that confer an anti-apoptotic function to latently infected cells. A prominent binding site for the human oncoprotein and chromatin regulator c-Myc has been charted in the EBER1 promoter, which may play an important role both in determining the chromatin structure of the viral genome and in the molecular mechanisms that lead to EBV associated malignancies, especially BL.

[1] Niller HH et al.: Acta Microbiol Immunol Hung **51**, 469-484 (2004).
[2] Niller HH et al.: Trends Microbiol **12**, 495-499 (2004).

CHARACTERIZATION OF ESBL PRODUCING HUMAN SALMONELLAE ISOLATED IN HUNGARY IN THE PERIOD OF 2000-2004

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Salmonella infection is a significant health problem worldwide. In many countries, the prevalence of human infections caused by multiple resistant *Salmonella* has been on the rise. In case of infections caused by multiple resistant strains, for infants, elderly or immuno-compromised patients, extended-spectrum cephalosporins are recommended for treatment. Consequently, since the 1990s, an increasing number of outbreaks and cases of infections caused by *Salmonella* resistant to extended-spectrum cephalosporins has been reported. The aim of the study was to screen the *Salmonella* isolates received by the Phage-typing and Molecular Epidemiology Department of the 'Johan Béla' National Center for Epidemiology during 2000-2004 for isolates resistant to the extended-spectrum cephalosporin cefotaxime (Ctx) and to characterize the CtxR strains by molecular methods.

Eight *S*. Typhimurium and one *S*. Enteritidis have been found to show cefotaxime resistance as determined by disk diffusion method. The MICs of the CtxR isolates for 11 antibiotic agents were determined by E-tests. The isolates were investigated by PCR using primer sets specific for *bla*TEM, *bla*SHV and *bla*CTX-M genes. The PCR products were sequenced. The integron carriage of the strains was also tested by PCR. The transferability of the antibiotic resistances and the identified extended spectrum b-lactamase (ESBL) genes have been tested by conjugational experiments; the clonal relationship of the strains was tested by pulsed-field gel electrophoresis (PFGE).
Five *S*. Typhimurium strains harboured the CTX-M-5 gene alone. The transconjugants obtained from these strains also had the CTX-M-5 gene, which was most probably transferred by an approx. 6.3 kb plasmid that was common in all transconjugants. Two *S.* Typhimurium strains had CTX-M-15 and TEM-1 genes, both of which were transferred to the transconjugants via an approx. 140 kb plasmid. These two strains plus one of the CTX-M-5 positive strains produced one or both out of the 1.45 kb and 2.0 kb integrons which have the gene cassettes of *aadB*-*catB3* and *oxa1*-*aadA1*, respectively. In a single *S*. Typhimurium strain, SHV-5 and TEM-1 genes were identified which were not transferable. This strain was integron negative. The CTX-M positive strains belonged to the same genetic clone; the SHV-5 positive strain represented a distinct clone. The *S.* Enteritidis strain possessed an SHV-5 gene alone, which was located on and transferred by an approx. 90 kb plasmid. A transferable 3.0 kb integron with five gene cassettes (*aacC4*-*aacC1*-*ORFX*-*ORFX'*-*aadA1a*) has also been identified in this strain. Cefotaxime resistance in the investigated *Salmonella* strains appears to link mainly to plasmid-mediated CTX-M or SHV type ESBL genes. The appearance of the CTX-M-5, CTX-M-15 and SHV-5 genes in *S*. Typhimurium as well as the appearance of an ESBL producing *S*. Enteritidis in Hungary is reported for the first time.

WATER DEMAND OF FUNGI AND POLLUTIONS WITH AEROALLERGENS

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"Water is the essence of all objects."
Thales from Milethos in Ion

Inhalation of air with >500 even inert particles/m^3 overloads the lungs, while fungi (all allergens) can pollute indoor air up to 10^4/m^3 (or higher) by a wealth of propagules from a "mycotation" in the environment [1]. This means an immense health risk, usually based on human factors. Fungi (as a biological system, BS) act as a closed dissipative thermodynamic systems forming a living system together with the relevant part of their environment (= vicinity, VIC). VIC nurses BS by nutrient support and excrement elimination. Water is privileged by fungi from the many factors of VIC (solvent, dispergent medium, nutrient, reactant, etc.), as they take up only dissolved small molecules (<4000 Da). Due to its high ΔGo pure H_2O is rare in nature, it is bound in solutions or hygroscopic systems as hydration agent with decreased vapour tension [Pv] and other parameters (a_w, Ψ, hyrel). Fungi use only the free, condensed portion of H_2O. Thus, H_2O is required in liquid form and with high percentage of free water molecules. The minimal value of water demand (minimal Cw in the substrate) of "true fungi" is 60% hyrel (or a_w = 0,6) (e.g. *Monascus bisporus*), while the maximum is 98% hyrel (a_w = 0.98) (e.g. *Schizosaccharomyces octosporus*) (*Hansenula* spp. are active at 90% hyrel, average bacteria at a_w 0.72 - 0.95). Within this range hydro- and hygrophilic, hydro- and hygrotolerant, hydro- and hygrophobic, xerotolerant, xerophilic groups are differentiated. The water requirement can be a "fixed value" or in a flexible range (homeio- or poikilohydrature group). The value is influenced by other VIC parameters, just as this alters the others. The sensitivity of various thallic organs and certain physiological processes (sporulation, sexuality, growth- and productive-, tropho- vs. idio-

phases) are different. Outdoor air-pollution depends mainly on objective, natural (geographic, climatic, etc.) conditions, while indoor air-pollution is influenced by human (social) interventions, and it is dependent usually on “illegitimate” H_2O sources (which are “facultative”, i.e. controllable). “Lodging – water – fungi” is the trias. The most important human indoor environment is lodging. In its panel or block type of industrial realization (suffering from errors in planning), and present day operation, results in undesirable humidity of the inside surfaces of front walls causing an enormous fungal colonization of the surfaces in question. It is rooted in the small cubature, the high number of lodgers, the high air humidity, poor ventilation, tight fitting windows and doors, poor heat insulation of walls (heat bridges, with well recognizable fungal growth) resulting in vapour condensation on cold wall surfaces (and also capillary condensation at lower vapour pressure).

[1] To the memory of Dr. Anna Csillag, the initiator and first head of the Mycology Department at "Johan Béla" National Centre for Epidemiology.

HALF-CENTURY HISTORY OF THE MYCOLOGY DEPARTMENT AT THE NATIONAL INSTITUTE OF PUBLIC HEALTH

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Fifty years ago, the Mycology Department was founded in the National Institute of Public Health for antibiotic research, but soon the objective changed to medical mycology as the new antibacterial agents provoked fatal systemic (deep) fungal infections. The unit started in a small laboratory under the leadership of Dr. Anna Csillag, a biologist trained in food mycology. She described a series of nosocomial secondary opportunistic mycoses of endogenous origin (candidosis, aspergillosis, cryptococcosis). Moreover, she developed a good fungal culture medium: the so called Csillag’s molasses-agar. She also detected potentially fatal fungal diseases (blastomycosis, coccidioidomycosis and histoplasmosis) in Hungary for the first time. Unfortunately, these were her last results in her homeland as she had to leave Hungary. Prior to her emigration, she summarized her relevant results in the *Acta Microbiologica Hungarica,* and discussed the destiny of the department with her chosen successor, a graduated microbiologist well-trained in mycology and biochemistry, having been recommended by dr. J. Bánhegyi, professor in mycology. Thus, the second period started with dr. Ervin K. Novák (as the head of the Deepartment) who, after some refinement and modernization of the workspace, apparatus and equipment pool and broadening of staff, could base the research on biochemistry, and introduce improved methodology in routine work. This renewal has gradually brought the Department to a considerable rank in microbiology-mycology, resulting in *bona fide* consequences: i./ initiation of the education of microbiology, mycology, biochemistry and related fields at 10 universities (including also classes of foreign students), soon with the promotion of dr. Novák (a “candidate” of science) to Titular University Professor, and ii./ assigning him the posts of Chairman (Mycology Section at the Hungarian Society for Microbiology) and Secretary (Biochemistry Section at the Hungarian Chemical Association) for repeated periods. The whole staff of the department took part at laboratory training. As a consequence, a school was started at the Department in the field of general and applied mycology for research fellows preparing dissertations to acquire bachelor, master or doctoral degrees. This period lasted until 1992, when Dr. Novák retired. On his proposal, the post was filled by J. Zala, a bioengineer (the present Head of the Department). She further increased the routine activity and capacity, while broadened the spectrum of serological methods. She succeeded in keeping the staff, despite of the decentralization and privatization of the medical mycology laboratory network and the alterations in institutional structure. During this half century, in collaboration

with "indoor and outdoor" colleagues, the following topics have been dealt with and combined into main projects (the number of published articles are given in parentheses): methodology (55), human mycoses (22), comparative physiology of axenic fungal cultures (72), taxonomy (72), cyto-serology (9), newborn thrush (6), growth kinetics and cell model (15), antifungals (48), hygienic mycology (91), chapters of handbooks and textbooks (36).

THE INITIAL STEPS IN LINCOMYCIN BIOSYNTHESIS

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Lincomycin A belongs to the group of clinically important antibiotics, however, our knowledge of lincomycin biosynthesis is still rather incomplete. Structurally, lincomycin A is composed of a sugar moiety, methylthiolincosaminide, and an amino acid derivative, propylhygric acid, linked by an amide bond. A pathway leading from tyrosine *via* L-DOPA to propylhygric acid was proposed based on the determination of the biosynthetic origin of carbon and nitrogen atoms using feeding experiments with subsequent NMR analysis. Sequence analysis of the *Streptomyces lincolnensis* lincomycin biosynthetic gene cluster revealed 29 ORFs with putative biosynthetic functions (*lmb* genes). *Lmb* genes were assigned functions in the biosynthesis of the aglycone moiety based on the results of transposon-mediated mutagenesis. The LmbB1 and LmbB2 proteins were described to transform tyrosine (LmbB2 and LmbB1) and DOPA (LmbB1) to an unidentified yellow product.
The LmbB1 protein was expressed in *Escherichia coli*, purified in its active form, and characterized as a dimer of identical subunits. Methods for purification and analysis of the LmbB1 reaction product were developed. Molecular mass and fragmentation pattern of the product revealed by capillary electrophoresis-mass spectrometry was in agreement with its proposed structure, 4-(3-carboxy-3-oxo-propenyl)-2,3-dihydro-1- *H*-pyrrole-2-carboxylic acid. The LmbB1 is thus a dioxygenase catalyzing the 2,3-extradiol cleavage of the DOPA aromatic ring, and the LmbB1 reaction product arises through subsequent cyclization of the primary cleavage product of 2,3-secodopa. A possible role of LmbB1 in 2,3-secodopa cyclization and alternative ways of the cyclization in the formation of biosynthetically related compounds, muscaflavin and stizolobinic acid, are discussed.
The LmbB2 protein was expressed in *Escherichia coli* and purified in its active form. By HPLC analysis of the LmB2 reaction mixture using DOPA as a standard, it was demonstrated that the LmbB2 protein is a monooxygenase catalyzing tyrosine hydroxylation to DOPA.

INVESTIGATION OF HUPK HYDROGENASE ACCESSORY PROTEIN IN *THIOCAPSA ROSEOPERSICINA*

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Thiocapsa roseopersicina is an anaerobic, phototrophic purple sulfur bacterium. There are at least two membrane-bound (HynSL and HupSL) and one soluble (HoxEFUYH) [NiFe] hydrogenases in the cells. The [NiFe] hydrogenases consist of large and small subunits. The large subunit contains the

composite binuclear metallocenter, which is the active site of the enzyme. The small subunit is responsible for electron transport between the active centre and the surface of the enzyme.
The maturation of these complex enzymes requires numerous accessory proteins, which are involved e.g. in metal incorporation or in the proteolytic cleavage of the C-terminal end of the large subunit. Tn5-based transposon mutagenesis was used to search for the hydrogenase accessory genes in this strain. Most of these auxiliary genes were found using this method, one of them was the *hupK* gene.
Very little is known about the exact function of the HupK protein. The homologous proteins possess some interesting features, for example they exist only in microorganisms which contain at least one membrane-bound hydrogenase, furthermore this type of protein is not present in *Esherichia coli*, whose hydrogenase is well characterized and the model for its assembly is widely accepted. The position of the *hupK* gene in *T. roseopersicina* is also interesting, it is not located as the last gene of the *hup* operon, as it is usual in other organisms, but it is found near the *hypC* and *hypD* accessory genes, irrespectively of the *hup* genes. In order to investigate the role of this protein, *DhupK* mutant strains were created. The mutant strains were characterized using hydrogenase activity measurement. The results showed that the activity of the membrane-bound hydrogenases decreased almost completely, while the soluble enzyme remained active. So HupK protein plays an important role in the formation of the functionally active membrane-bound [NiFe] hydrogenases, but not in the biosynthesis of the soluble enzyme. More detailed information can be obtained about the role of HupK on the protein level. Special expression vector was used to produce active HupK protein in homologous host. Protein partners - interacting with HupK during the hydrogenase assembly - can be fished using this vector under appropriate conditions.

COMPARATIVE GENOMICS OF DIFFERENT STRAINS OF *WOLBACHIA PIPIENTIS* BACTERIA

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We report the results of our efforts to characterise and analyse the available genome sequences of the endosymbiotic alpha proteobacteria *Wolbachia pipientis* including the following strains: wUni, the parthenogenesis inducing endosymbiont of *Muscidifurax uniraptor* parasitic wasp and wRi, the cytoplasmic incompatibility inducing endosymbiont of *Drosophila simulans* Riverside. No genome size has previously been estimated for wUni but for wRi PFGE data suggested a size of 1.66 Mbps, and the genome size of wMel, the cytoplasmic incompatibility inducing symbiont of *Drosophila melanogaster*, has been correctly estimated by PFGE and later corroborated by full sequencing to be the size of 1.26 Mbps. To sequence the wRi genome, a whole genome shotgun library was constructed using a modified M13 vector. For wUni it was impossible to construct a shotgun library because of insufficient DNA material originating from low infection level of the symbiont. Therefore a combined PCR and LPCR approach was used. The primers for the PCR were designed based on the available wMel genome sequence data and the PCR products were directly sequenced. LPCR products were shared and blunt-end cloned into pcSmartHCKan vectors. All sequencing was performed using the MEGABace system. Collected data were processed using the Phred-Phrap package. The wRi genome is now in the gap closure phase, after a collection of 1.3 Mbp sequence data assembled into 61 contigs longer than 3000 bp. The assembly of the wUni genome at the moment contains 610329 bps from 3139 reads divided into 382 contigs. The gene order structure between these ge-

nomes is quite well conserved and the overall sequence identity is very high. The size differences and the differences in the observed phenotypes of the infections could possibly be explained with differences in the genomes, including, for example, the number and location of transposons and ankyrin repeat proteins. Differences and similarities of the listed Wolbachia genomes are discussed here. We also try to correlate specific genomic content with the observed diversity of biological characteristics and effects shown by the different strains.

IMPLEMENTING THE MULTIPLEX PCR METHOD IN DETERMINING RESISTANCE TO ISONIAZID IN *MYCOBACTERIUM TUBERCULOSIS* STRAINS IN CROATIA

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Croatia has a tuberculosis (TB) incidence of 28/100,000, with an incidence of anti-TB drug resistance of 1/100,000, and that of 0.3/100,000 of multidrug resistance (MDR). MDR-TB strains are defined as strains resistant to at least rifampin (RIF) and isoniazid (INH), with or without resistance to other drugs. The routine drug susceptibility testing method used in Croatian National Mycobacteria Laboratory is the conventional proportion method on Lowenstein-Jensen medium.
At the moment, isoniazid is the most effective antituberculous agent available. The genetic basis of INH resistance in *M. tuberculosis* has been attributed to at least two different genes. Deletion and mutations of the *katG* gene encoding catalase-peroxidase have been shown to cause resistance to INH. Catalase-peroxidase converts INH to an active form, which affects mycobacterial proteins (such as InhA) that are required for the synthesis of mycolic acids. Mutations of the *inhA* operon have been associated with INH-resistant *M. tuberculosis* isolates. Recently, a new multiplex PCR method for the rapid detection of INH resistant *Mycobacterium tuberculosis* clinical isolates was introduced. The method is based on allele-specific PCR methodology targeting an AGC to ACC mutation in the *katG* gene and an *inhA*C-15T mutation in the regulatory region of the *mabA-inhA* operon, to detect INH-resistant *M. tuberculosis* strains in the same PCR tube for each sample. A total of 80 isolates, collected from 1999 to 2004 in Croatia, were investigated and identified as INH-resistant *M. tuberculosis* strains by standard susceptibility testing methods. Susceptibility testing with INH (0.2 and 1.0 μg/ml) was performed with Lowenstein-Jensen medium by the proportional method of Canetti. A total of 15 *M. tuberculosis* strains that were susceptible to INH were used as controls.

CLONING AND CHARACTERIZATION OF FPAC1, AN ADENYLATE CYCLASE GENE FROM *FUSARIUM PROLIFERATUM*

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Adenylate cyclase is a membrane-bound enzyme in eukaryotic cells and catalyzes the conversion of ATP to cyclic AMP on the inner side of the plasma membrane. cAMP acts as a secondary messenger and activates cAMP-dependent protein kinases. These kinases transfer phosphate groups to other specific proteins and the phosphorylated substrates take part in regulation processes. In fungi examined

until now (e.g. *Neurospora crassa*, *Candida albicans*, *Cryptococcus neoformans*, *Magnaporthe grisea*), adenylate cyclase plays an important role in mycelial growth, conidial germination, pathogenicity and mating.
The present investigation was aimed at cloning and characterization of an adenylate cyclase encoding gene, *Fpac1* from *Fusarium proliferatum* (teleomorph: *Gibberella fujikuroi*, MPD-2). Degenerate oligonucleotide primers were designed for conserved sequences of adenylate cyclase genes known from other filamentous fungi. The PCR product hybridized to a single band on the genomic DNA gel blot, indicating that the cloned DNA fragment originates from *F. proliferatum* and exists as a single copy in the genome. The entire copy of *Fpac1* was isolated by screening the genomic library of *F. proliferatum* strain ITEM 2287 using the PCR product as a radioactive probe. Functional analysis of the *Fpac1* gene was carried out using a gene disruption strategy. The putative catalytic domain of *Fpac1* was replaced by a hygromycin resistance gene cassette and *F. proliferatum* protoplasts were transformed with this gene disruption construct. Homologous recombination occurred in four of the 54 stable hygromycin resistant transformants. All four *ΔFpac1* mutants showed reduced vegetative growth, delayed conidial germination and distorted germ tube shapes. In mating experiments, when the mutants were used as female partners, significant reduction in the number of perithecia was observed after five weeks of incubation. *F. proliferatum* ITEM 2287 is a self-incompatible strain. Interestingly, the *ΔFpac1* mutants were able to overcome this self-incompatibility. These results confirm that the adenylate cyclase gene is not vital for growth under optimum conditions, but essential for adaptation to a new environment and plays an important role in stress response and recombination events of plant pathogenic fungi.
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MEASUREMENT OF BIOLOGICAL ACTIVITY OF DIFFERENT SOIL TYPES

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The biological activity of six different types of soil originating from diverse places and involving different cultivation methods was measured. Three different measurement methods were applied to determine the approximate biological activity of soils; these were the measurement of the total number of soil bacteria, of the activity of some soil enzymes, and of CO_2-production. These three methods may inform on the biological activity of soils having different physical and chemical properties and humus content. It seems that the total number of bacteria, the number of aerobic N_2-fixing, nitrifying and aerobic cellulose decomposing bacteria generally were higher in cultivated soils than in the undisturbed (non cultivated) soils. The number of microscopic fungi correlated with the pH of the soils, a larger count was measured in case of brown forest soil. The activity of dehydrogenase and urease enzymes were influenced mainly by the texture and organic matter content of the soil, the highest enzyme activities and CO_2-production were measured in the undisturbed brown forest soil and in the marshy meadow soil. It can be stated that beside the differences in soil quality - the texture of soil, humus content, pH, etc, - several agrotechnical factors also significantly affected the soil bacterial count, and the enzyme activities studied (dehydrogenase and urease). The different cultivation methods affected the biological activity of soils having similar properties. It was experienced that seasonal fluctuation of biological activity of the soil strongly depends on the colloid content; bigger seasonal changes were measured in soils having a smaller colloid content.

ALTERATION OF CYTOKINE PATTERN IN CD4 T LYMPHOCYTES BY HUMAN HERPESVIRUS 6B INFECTION

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Both A and B variants of human herpesvirus 6 (HHV-6) and HHV-7 establish latency in CD4 immune cells, but upon reactivation they induce different disorders. These might –probably- depend on different mediator patterns produced by the infected lymphocytes. Following studies on cytokines induced by HHV-6A and HHV-7, recently we have investigated cytokine production of CD4+ MOLT-3 cells during the complete course of viral replication cycle. HHV-6B was concentrated by ultracentrifugation, then cells were infected at high multiplicity for synchronized infection. Supernatant and cell samples were collected at different time intervals until the cells died. Cytokine secretion was measured by sandwich ELISA while the intracellular mRNA content by real time RT-PCR. Mock infected cells were controls. It was found that, HHV-6B first increased subsequently decreased interleukin (IL)-2 and interferon (IFN)-γ production; its effect on IL-12 secretion was the opposite. Disintegration of their synergism leading to immune suppression might be augmented by increased level of tumour growth factor (TGF)-β1. Diminished production of IL-15 inhibits activity of natural killer cells. Severe suppression of granulocyte-monocyte colony stimulatory factor (GM-CSF) and IL-3 secretion might disturb B cell maturation leading to lymphomagenesis. Diminished IL-4 secretion results in weak antibody production and anti-tumour activity, while suppressed IL-10 level retards B cell maturation. Low levels of TNF-α and β explain why HHV-6B is not able to activate human immunodeficiency virus (HIV). High IL-1β level might be responsible for high fever in acute herpesvirus infections. HHV-6B seems to affect both Th1 and Th2 cytokine systems. Acute diseases normalise rapidly but chronic infections might be devastating.
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LOW RATE CARRIAGE OF ENTEROVIRULENT *ESCHERICHIA COLI*

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For common enteric pathogens, like salmonella, shigella or the vibrios, selective enrichment media are available and likely colonies of the pathogens are easily spotted on differential plates. Quantitative interpretations of the findings are seldom applied, i.e. once any number of colonies of these pathogens is detected, the finding is usually considered significant from a clinical, as well as from an epidemiological point of view. For most pathotypes of enterovirulent *E. coli*, enrichment media are not available and these strains seldom express phenotypes helping their easy identification. Consequently, they are usually identified with molecular assays investigating 3 to 10 randomly selected colonies. While this approach may reveal infections assumed to result in a high rate carriage of the pathogen (e.g. over 10 percent of all coliforms), no data are available about the possible presence of these pathogens in low ratios in the fecal specimens of healthy individuals and in patients with diarrhea.

Using PCR assays specific to the various pathotypes of enterovirulent *E. coli*, the DNA extract of mixed coliform cultures of 100 healthy adult individuals and that of 106 diarrhea patients were tested. Two hundred colonies, from each of the positive samples, were further tested with PCR to identify the pathogens. Genes of enterovirulent *E. coli* were detected in 32% of the samples of the sick, and in 40% of the healthy people, while the isolation rate of the pathogens was 22.6% and 26%, respectively. Enteropathogenic (EPEC) (11.3% in healthy and 25% in diarrhea patients) and enteroaggregative *E. coli* (EAggEC) (12.2% in healthy and 9% in diarrhea patients) were the most frequently encountered groups. However, 4.7% of the patients with diarrhea carried EPEC <5%, and the same number of patients in a ratio <0.5%, compared to the normal coliform flora. The corresponding figures for healthy people were 12% and 9 %, respectively. Less than 5% of the coliforms in the stool samples of 7.5% of the sick and 2% in those of the healthy people were identified as EAggEC, and a further 0.9% and 1% carried the pathogen in a ratio below 0.5%.
These data show that particularly EPEC and EAggEC are frequently carried by both sick and healthy people in a ratio below the detection limit of molecular assays investigating a few representative colonies only. The significance of this low rate carriership in the development of clinical diseases is not known and needs further investigation. However, it is suggested that these low rate carriers may play a role as sources of infections and in maintaining the pathogen in the host population.

POSSIBILITIES OF BIOETHANOL PRODUCTION APPLYING TUBER CROPS AS RAW MATERIALS

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The production of alcohol from plants is one of the oldest biotechnological processes. Many industrial applications need ethanol such as the food industry: in the preparation of potable drinks and the production of vinegar. Ethanol is an important organic solvent in the chemical industry for many cosmetic and pharmaceutical applications, as well as being exploited as biofuel. Therefore, a continuous search is going on to find cheap, readily available raw materials for bioethanol production. Our studies included a comparison of Jerusalem artichoke with well-known crops from an agronomic point of view. The main aim was to determine the industrial potential of Jerusalem artichoke for ethanol production. Nowadays, Jerusalem artichoke can be found in small plots throughout Hungary. It is recently rediscovered as a distinct food, feed, and raw material for the industry and as an energy crop; its large-scale production is therefore recommended and expected. At present, Jerusalem artichoke is even being considered as the pre-eminent raw material of the future. Jerusalem artichoke contains a high quantity of inulin, but potato and sweet potato (batata) have starch as storage carbohydrate. Therefore, different mashing processes were used to prepare wort, and yeasts applied in the fermentation processes were also distinct. Amylolytic enzymes (alfa-amylase and glucoamylase) were used to prepare wort in the case of batata and potato, and fermentation was carried out with *Saccharomyces cerevisiae* strains (UVAFERM CM and 228). When Jerusalem artichoke was the raw material, Novozym inulinase was used for wort preparation and fermentation was initiated by a *Kluyveromyces marxianus* strain. Yeasts of the genus *Kluyveromyces* are well known for their ability to grow on fructans like inulin. *Kluyveromyces marxianus* was selected for the study of parameters relevant for the commercial production of inulinase.
To get more detailed information, various methods were applied to get fermentation media. The pH and dry matter content of all media were adjusted to 3.5 by HCl and 130 g/L, respectively. The fermentation medium was a non-sterilized pulp or juice of Jerusalem artichoke and was massively pitched. Fermentations were run in stainless steel fermentation tanks with a working volume of 18 li-

tres at 24 °C for 6 days. The fermented materials were distilled by means of a pot style distillatory and the fractions of the distillate were collected and analysed. The composition of spirit fractions originating from various raw materials was very different. The heart fractions of sweet potato and potato were neutral. The esters and higher alcoholic components were located in the head fractions. The spirit from the juice of Jerusalem artichoke contained high ethanol concentration, low ester and higher alcohol contents. It can be concluded that, when applying well-controlled technologies, all of these materials ensure distinct, but good quality ethanol products.

RAPID IDENTIFICATION OF MICROORGANISMS IN BLOOD CULTURES USING FLUORESCENT IN SITU HYBRIDIZATION

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Rapid identification of the causative pathogen in septicaemia is crucial. With the use of fluorescent in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes, pathogens are rapidly identified in positive blood culture bottles before subculturing on agar plate and biotyping. The aim of this study was to evaluate the usefulness of fluorescent in situ hybridization of microorganisms grown in blood culture specimens. A total of 100 bacteria obtained from blood culture were evaluated. A total of 73 bacteria could be identified by FISH on family, genus or species level within 3 hours. Most of the obtained organisms were coagulase negative *Staphylococci* (N. 48). Almost all of them could be identified, except for one. However, in that case the fluorescent signal was not on the bacteria. We were able to identify 9 from 13 isolates of *Staphylococcus aureus* 1 day earlier. In this group there were 2 MRSA isolates. In 3 immunocompromised cases, we were able to differentiate between *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* 1 day earlier. In 13 cases, we did not choose appropriate probes, but in these cases there were not any false positive signals. In only 9 cases there was no signal despite the use of adequate probes. Our oppinion is that FISH assay is an appropriate method for the rapid identification of pathogens grown in blood cultures.

BIOREMEDIATION OF CHLORINATED BENZENE-CONTAMINATED SOIL

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Chlorinated compounds are synthesized by the chemical industry in large quantities and their release into the natural environment has created major pollution problems. Microorganisms are, however, highly adaptable to changes in the environment and have consequently evolved the genes encoding enzymes capable of degrading the chlorinated compounds to varying degrees. Chlorinated aromatic compounds, in particular the heavily chlorinated benzenes, biphenyls and dioxins, are among the most problematic environmental pollutants because of their chemical inertness, lipid solubility, and high toxicity. Most members of these classes of compounds are listed as priority pollutants by the environmental agencies of Europe and the USA. Naturally occurring microorganisms have evolved pathways to degrade and mineralize some, but definitely not all, of these compounds. Numerous microorganisms have been isolated which – alone or in consortium - could degrade most of the chlorinated benzenes, but the most heavily chlorinated compounds such as hexachlorobenzene, pentachloro-

benzene, and 1,2,4,5-tetrachlorobenzene, as well as the less chlorinated molecules 1,2-dichlorobenzene and 1,3,5-trichlorobenzene are particularly resistant to biodegradation.
It is well known that the region surrounding Garé, a small village in South-Hungary was highly contaminated with halogenated benzene derivatives. Highly contaminated soil was transferred and burned at extreme high temperature. However, this strategy was not applicable for larger areas containing less pollutants and a biological approach seemed to be a reasonable solution.
Our aim was to isolate single strains or a microbial community capable of degrading both perchlorinated and less halogenated benzene derivatives in samples derived from the Garé area. For the bioremediation process we preferred to apply the bioaugmentation and biostimulation processes. Finally, bacteria capable of converting these compounds could be isolated. Partial taxonomical identification of the strains revealed that all of them belong to the *Pseudomonas* genus. Optimization of the bioremediation process on lab scale and *in situ,* is in progress.

DISTRIBUTION OF *SALMONELLA* ENTERITIDIS PHAGE TYPES IN CROATIA

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Salmonella phage-typing, as one of the oldest epidemiological markers, has been in use for more than 50 years- applied at first mostly for S. Typhi, S.Typhimurium and S.Paratyphi B. However, in the late eighties of the 20th century S.Enteritidis became to be the prevalent serotype in most European countries-except for the Scandinavian Peninsula. So, dr.Linda R.Ward and co-workers selected 10 bacteriophages (1987.); 34 different phage types could be distinguished with their use. The same authors introduced 6 new phages (1997.), all together 16 that enabled to discriminate 74 phage types (PTs).
As of June 2004, the new scheme for phage-typing is used at the National Centre for Salmonella.
Two randomized samples of S.Enteritidis isolates are included in this study: 119 isolates (93 human isolates, 26 of animal or food origin). Sample A: i. among human isolates the prevalent PTs are: 5a, 21 and 16; ii. among non-human isolates the most frequent PTs are: 4, than also 21 and 16; iii. only 1 isolate is untypeable. Sample B: i. among the only 8 human isolates 4 are PT 4b; ii. among non-human isolates the prevalent PTs are: 4b and 18; iii. 4 isolates are untypeable.
PT4, which predominated Europe until the middle nineties, has never been the most frequent phage type among human S.Enteritidis isolates in Croatia, although it occured in several outbreaks.

SMALL-SCALE BIODEGRADATION EXPERIMENT FOR BIOREMEDIATION OF POLYCHLORINATED BIPHENYL-CONTAMINATED SOIL

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The main objective of our study was to propose a remediation method for enhancing PCB transformation in contaminated soil of the transformer station TS 110/35 kV Zadar, Croatia, damaged during warfare operations in 1991. Two fundamental approaches for enhancing biodegradation of accumulated PCBs in contaminated soils have been comprised in our small-scale biodegradation experiment, i.e. i) stimulation of the growth of contaminant degraders within indigenous soil microflora (biostimulation) and ii) introduction of microbial cultures of known PCB-degradation capacity (bioaug-

mentation). Two cultures were chosen as seed cultures: i) mixed culture originating from PCB-contaminated soil of the transformer station area (TSZ7) and ii) a pure culture isolated from this mixed culture (strain Z6, identified as *Rhodococcus erythropolis* using 16S rDNA gene sequence comparison). These cultures showed substantial growth and PCB-degradation activity in the presence of biphenyl as supplemental carbon source.

Based on growth kinetics studies of the selected cultures and biotransformation experiments in which the factors that affect PCB biodegradation were studied, a plan for a small-scale-field experiment was established. It comprised of three pots filled with well homogenized soil of the transformer station. Each pot was equipped with a small irrigation system operated by a peristaltic pump. Plots were divided into two parts; one was filled with earthworms *Lumbricus rubellus (Annelida, Oligochaeta, Lumbricidae)*. Earthworms were used to improve the dispersal of soil inoculants, to transport microbial inoculants into deeper layers and to increase soil aeration. To facilitate natural changes of environmental conditions, experimental pots were held in a semi-opened room (protected from rain and wind). The soil PCB bioaugmentation treatment employed repeated inoculations with the selected cultures along with the addition of surfactant soya lecithin, for enhancing PCB bioavailability (1 g/kg), xylose as supplemental carbon source (1 g/kg) and carvone as inducer of PCB catabolism (50 mg/kg). The biostimulation treatment included only the addition of carbon source, inducer and surfactant. Amendment and inoculation of the soil was carried out weekly to maintain augmented culture density as well as soil moisture. Soil samples were collected every month and analysed for PCBs by capillary gas chromatography with mass spectrometric detection. The changes in PCB congener mass fractions in soil were quantified against a standard solution of Aroclor 1248. Gradual decrease of PCB mass fractions in soil observed in all experimental pots, and a slightly higher decrease of some PCB congeners in the pots bioaugmented with xylose-grown, carvone-induced, mixed bacterial culture TSZ7 during a four-month treatment is considered as a promising indication of partial soil decontamination. Furthermore, a simple design of our small-scale-field experiment should facilitate its application in field conditions.

INDOOR MYCOLOGY NETWORKING IN NEW EU MEMBER STATES FROM CENTRAL EUROPE

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Moulds growing in damp indoor environments may produce harmful pollutants: propagules, allergens, toxins and volatile irritative compounds. Health effects include repeated respiratory infections, irritation of mucous membranes as well as irreversible damages such as asthma and allergy. Children are the main risk group.

Coordination of management of indoor fungal contamination is best developed in Scandinavia and North America where several networks perate, e. g. the Nordic European Program NORDDAMP. Based on the experience of the internationally certificated indoor mycological laboratory of the Slovak Medical University Bratislava (SMU) over the last decade, dissemination and sharing of knowledge and experience on indoor fungal management in the new EU member states (NMS) and its linkage to the existing programs should be of value. Therefore, the establishment of the network on recognition and management of indoor fungal exposure in dwellings and public buildings regarding its

health effect has been one the objectives of an EU funded project "Improving environmental health research and management in newly associated states running in the SMU in the context of the Quality of life and management of living resources RTD Program, FP5 key action Environment and health" already running for 2 years.
The partners involved, from 5 Central European countries, have completed a survey of: - the extent of contamination of dwellings by moulds; - mycological, physical and chemical investigation in affected houses; - medical investigation of their occupants with/without ill-health problems; - methods for prevention of fungal and damp exposure in buildings; - information and education of the public about the measures necessary to prevent and/or control indoor fungal contamination, incl. economic costs and benefits of prevention vs. therapy. Currently, the audit made it clear that the state of art on exposure, assessment, analytical methods, epidemiology, health effects, prevention and remediation of fungi and their metabolites in the indoor environment in NMS is generally limited. However, some countries (Poland, Lithuania, Slovakia) have established quite serious research in this field, and there are particular hygienic limits for indoor mould contamination set down in all of them. Medical investigation and/or dispensary of mouldy house inhabitants seems to be the weakest point of the complex management of health and economic losses due to fungal and moisture exposure in buildings. A project workshop on strategy in indoor fungal research and prevention is under preparation. The network is open to every interested partner.

SUSCEPTIBILITY OF *ASPERGILLUS* SECTION *NIGRI* TO ITRACONAZOLE DETECTED ACCORDING TO NCCLS

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Species of *Aspergillus* are currently isolated from foods and used in industry, for example for citrid acid production. Some of them synthesise a carcinogenic mycotoxin – ochratoxin A. They are usually involved in chronical otitidies (St–Germain and Summerbell, 1996), perithonitis and endophtalmitis, endocarditis, skin and disseminated infections. *A. niger* is causing about 5% of clinical mycoses in humans, so it belongs to the most frequent fungal agents.
Testing of sensibility and values of minimal inhibitory concentration – MIC *in vitro* indicate that itraconazol is effective against a wide range of clinically and environmentally interesting species of fungi. From the whole spectrum of effectiveness, the effect against species of *Aspergillus* is the most significant one. So far, species of *A. flavus, A. fumigatus, A. nidulans, A. niger* and *A. terreus* have been tested for their sensitivity/resistance to itraconazole by several methods (Haber and et., 1995).
A. niger values MIC50 (minimal inhibitory concentration of itraconazole that inhibited growth of 50% colonies) was established at 0.5 μg/ml and MIC90 (minimal inhibitory concentration of itraconazol that inhibited growth in 90 % of colonies) at 1.00 μg/ml (on RPMI 1640 medium, by NCCLS microdilution or macrodilution methods).
We have applied the NCCLS document M38 – P Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi, proposed standard. The method is intended for testing the more common conidium-forming filamentous fungi that cause invasive infections. These moulds encompass *Aspergillus* species, *Fusarium* species, *Rhizopus* species*, Pseudallescheria boydii* and the mycelium form of *Sporothrix schenckii.*
16 environmental *A. niger* strains (soil origin) resistant to amphothericin B (tolerance up to10 μg/mL) were grown on potato dextrose agar (PDA) slants at 35°C for 7 days. Inoculum was formed by conidia and the concentration of the inoculum was 0.09 – 0.11, for standard medium 0.4 – 5 x 10^6 cfu/mL, a final concentration of inoculum was 0.4 – 5 x 10^4 cfu/mL. As cultivation medium RPMI

1640 was used,, 0.03% glutamin, 0.165 M MOPS (morpholinpropansulpfan acid), 2% glucose, pH 7.0 + 0.1, a concentration of itraconazole (Janssen Pharmaceutica, Beerse) 0.015 – 16 µg/mL, incubation at 35°C for 46 to 50h was applied. Detection was evaluated visually, 0 –absence of growth, 1 – 25% of the growth control, 2 – 50% of the growth control, 3 – 75% of the growth control and 4 – no reduction in growth. The used microdilution method on PDA at incubation temperature 35°C confirmed inhibitory influence of itraconazole on all species of *Aspergillus* section *Nigri.* All the strains tested showed resistance to itraconazole under given conditions, as they were able to tolerate concentrations of even 5.0 µg/mL. It is clear from these results that standardized methods for susceptibility testing of filamentous fungi against antimycotics is important, as is the development of new medicament form (solution of itraconazol with cyclodextrin) and synthesis of less toxic and systematically effective antimycotics (e. g. voriconazol, saperconazol, etc.).

ARE THE HUNGARIAN DRINKING WATERS CONTAMINATED WITH *GIARDIA* AND *CRYPTOSPORIDIUM* PROTOZOONS?

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The presence of *Cryptosporidium sp*. oocysts and *Giardia lamblia* cysts in waters is an increasing problem worldwide. Several outbreaks of cryptosporidiosis and giardiosis have already been documented. Cryptosporidium is a coccidian protozoan parasite and it has a complicated life cycle that goes through many forms. The most relevant form is the oocyst, which contains the infective sporozoits. Giardia is a flagellated protozoan parasite and it exists in two distinct morphological forms. The most relevant form is the cyst, which is the infective stage. Diarrhoea with vomiting and general weakness are the symptoms after infection. An important mode of transmission to humans is via contaminated drinking water or recreational water. Wildlife and sewage outflows have been implicated in watershed contamination and farm animals are also believed to be major contributors. Both organisms are characterised by the ability to survive in aquatic environments and have a high tolerance to many drinking water disinfectants. Conventional water treatment procedures do not remove or inactivate these protozoa properly, only special methods do. These methods are absent from most of the treatment plants. The method of USEPA (1623) was introduced for the detection of Giardia and Cryptosporidium in water. Filta-Max foam filters were used for the first concentration step and 100-1000 litres of finished water was filtered. In case of raw water,, if water turbidity was too high, chemical flocculation was carried out for oocyst and cyst recovery from 4-20 litres of sample. After the first concentration, oocysts and cysts were isolated using immunomagnetic separation (IMS). This technique involved the attachment of oocysts and cysts to magnetic particles coated with anti-Cryptosporidium and anti-Giardia antibodies. The oocysts and cysts were separated from interfering materials within the water concentrate using a magnet. After IMS, organisms were stained with fluorescent-labelled monoclonal antibodies (FITC) and nucleic acid stain (DAPI). Each sample was then examined using epifluorescence and differential interference contrast microscopy. All the endangered drinking water resources and finished water were examined with the methods mentioned above; all together 62 samples, 25 from raw water and, 37 from drinking water, respectively. Until now, 7 raw water pools were contaminated with Giardia, 4 with Cryptosporidium and 7 drinking water samples were contaminated with Giardia, 5 with Cryptosporidium. The highest level in drinking water was 0,4 cyst/10 litres and 0,3 oocyst/10 litres for Giardia and Cryptosporidium, respectively. The highest level in raw water was 10^3 cysts/10 litres and 3 oocysts/10 litres of Giardia and Cryptosporidium, respectively. 99 % of the identified cysts or oocysts were empty (we could not see the nuclei stained

with DAPI). In the presentation, you can hear about the accurate protozoon numbers in different kinds of water treatment plants and about their removal technologies.

COMPARATIVE STUDIES ON BACTERIAL COMMUNITIES FROM SEDIMENTS OF SODA LAKES LOCATED IN THE REGION OF TISZÁNTÚL, HUNGARY

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Soda lakes are naturally occurring, highly alkaline and usually saline environments, which can be characterized by large amounts of Na_2CO_3 and the lack of Mg^{2+} and Ca^{2+} ions. These extreme environments provide great microbial diversity, and are among the most productive environments on earth. This study was aimed at revealing the microbial diversity of the extremely shallow, alkaline and moderately saline soda lakes of the Hungarian Tiszántúl by applying cultivation based and cultivation independent methods.
Samples of sediments from soda lakes of Fehér-szik and Nagy-Vadas-tó were obtained in 2004 and 2005. Cultivation based studies were grounded on isolated bacterial strains, which were investigated for their morphological, biochemical and ecological tolerance characters and grouped by hierarchical cluster analysis. Selected representative strains were identified by 16S rDNA sequence analysis. Cultivation independent methods such as cloning and denaturing gradient gel electrophoresis (DGGE) were also used in order to have a complete understanding of the diversity of the Tiszántúl soda lakes.
Characterization of 98 isolated strains for 26 biochemical and physiological features proved that the majority of the strains were Gram positive (80%) and rather metabolically inactive. According to the NaCl and pH tolerance tests, most of our strains proved moderately halophilic and alkalitolerant with a few specific alkaliphiles. The cluster analysis resulted in two distinct phena (with min. 80% similarity), although one third of the strains formed single member phena. Phylogenetic analysis of the cluster representatives revealed the highest similarity to the alkalitolerant and halotolerant species *Nesterenkonia halobia* and various obligatory alkaliphilic *Bacillus* species. Phenotypically variable, unclustered strains showed real phylogenetic diversity. The majority of the strains gave a low percentage of 16S rDNA similarity, thus these strains can be assumed as new species.
Cultivation independent methods showed a quite different image. Our clone library resulted in mainly Gram-negative species with low percentage in sequence similarity. Majority of the clones proved to be members of Proteobacteria and uncultured Gemmatimonadetes, while a few Actinobacteria, Sphingobacteria, Chlorobi, Deinococcus-Thermus, Chloroflexi and Nitrospirae species were also detected. Using DGGE, we could compare the DNA extracts from both lake sediments, taken in spring, summer and autumn 2004. According to DGGE, constant communities seem to inhabit both lakes, only the dominance ratios change with the differing ecological factors. The most characteristic bands were identified as *Serratia*, *Nesterenkonia*, *Aeromonas, Spirochaeta*, *Amycolatopsis*, *Halothiobacillus* and *Chloroflexi* species. Cultivation independent methods revealed numerous anaerobic and chemolitotrophic species, which were impossible to cultivate with the methods used. A few sequences confirmed the results of cultivation; e.g. the genus *Nesterenkonia* was represented using each method, validating our hypothesis that this genus must be dominant in the sediment communities of the investigated soda lakes.
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POPULATION DYNAMICS OF SACCHAROMYCES SENSU STRICTO DURING FERMENTATION OF BOTRYTIZED ASZU WINE

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However numerous studies have examined the microbial diversity of botrytized wines, our knowledge about the Tokaji Aszu fermentation and the participating yeast species is limited.
The aim of the present work was to examine the fermentation kinetics; to isolate *Saccharomyces* yeasts from fermenting aszu wines; to identify the isolated strains on species level; and to study the population dynamics of *Saccharomyces sensu stricto*.
Three experimental fermentations of aszu wine were followed. In the case of fermentation No. 1, a spontaneous fermenting must was used for the extraction of aszu-berries, while in the cases of No. 2 and No. 3, the extracting medium was fermenting must and wine, respectively, which were inoculated with a *Saccharomyces cerevisiae* killer starter strain. The fermenting wines were analysed for chemical composition. *Saccharomyces* strains were isolated at eleven different points in time of fermentation by using a selective plating method. The isolates were identified by rDNA RFLP analysis. The RFLP patterns obtained were compared with the ARDRA molecular databank. RAPD method was used for the examination of diversity at subspecies level and for monitoring the starter strain during fermentation. The fermentations were conducted for six months. The kinetic results showed that in the case of No. 1, the fermentation started immediately after extraction and was slow, while in the cases of No. 2 and No. 3 the process was delayed for almost three months, but after the start ethanol concentration increased rapidly. In spite of these differences in fermentation kinetics, when the chemical compositions of the produced aszu wines were compared, there were no significant differences.
A large number of *Saccharomyces* isolates were collected. Their identification by ARDRA clearly showed that *Saccharomyces cerevisiae, S. bayanus var. bayanus* and *S. bayanus var. uvarum* strains took part in fermentation, however, in the case of some strains a mixed ARDRA pattern of *S. cerevisiae* and *S bayanus* was observed. The incidence of *S. bayanus* was very high, it was dominant even in fermentation No.1, where *S. cerevisiae* was represented by a smaller population. Frequency of *S. cerevisiae* was the highest in the case of fermentation No. 3 (61%), which was almost three times higher than in No.1. In each fermentation, molecular diversity was very wide at subspecies level *S. cerevisiae* was more polymorphic than *S. bayanus*. In the case of fermentations No.1 and No. 2 the starter strain rarely occurred, while it was found in high ratio in No. 3.
We may conclude that the starter strain used for fermentation of extracting must or wine influences the fermentation kinetics, as does the population dynamics of yeasts during the second fermentation of aszu wine. The starter strain can survive in crude aszu and changes can occur in the ratio of the fermenting *Saccharomyces* populations.
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ISOLATION AND MOLECULAR BIOLOGICAL INVESTIGATIONS OF AVIAN POXVIRUSES FROM CHICKEN AND TURKEY IN CROATIAN FLOCKS

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Fowl pox is a common disease of chicken and turkey with economic importance to the poultry industry. Disease prevention is achieved by vaccination with live fowl poxvirus (FPV) or pigeon poxvirus. In recent years, numerous outbreaks have been reported in vaccinated flocks, suggesting that these vaccines are often not effective. Several hypotheses on the possible causes of re-emergence are discussed, beside the possible alternation in the pathogenicity and virulence of recent isolates, speculation on the modification in the biological phenotype of FPV due to the integration of Reticuloendotheliosis virus (REV) in their genome were published. Because REV has been associated with immunosuppression, its presence in the genome of FPV seems to play an important role in the pathogenesis of fowl pox, and prolongs persistence of FPV in the bird population.
In 2002, several outbreaks of avian pox were observed in North Croatia. Four strains of avian pox viruses were isolated from cutaneous lesions by inoculation of embryonated chicken eggs on the chorioallantoic membranes (CAM). The resulting proliferative CAM lesions contained eosinophilic cytoplasmic inclusion bodies. One isolate originated from a meat turkey flock and two strains were isolated from layers. The fourth originated from a small pigeon population of the same area.
Further identification and differentiation of the four various avian poxviruses were carried out by the use of polymerase chain reaction (PCR) combined with restriction enzyme analysis (REA). Using one primer set, which framed a region within the fowl poxvirus (FPV) 4b core protein gene, we were able to detect avian poxvirus specific DNA from all four tested isolates. PCR results revealed no recognizable difference in the size of amplified fragments among the different avian poxviruses from chicken, turkey and pigeon. Restriction enzyme analysis of PCR products using *Nla*III showed the same cleavage pattern for turkey and chicken isolates and a different one for the pigeon isolate.
The pathogenicity of these four poxviruses from turkey, hen and pigeon was unusually high causing mortalities between 29% (turkey) and 10% (pigeon). Further examinations were carried out to determine the possible integration of Reticuloendotheliosis virus (REV) in the genome of isolated avian poxviruses. For this purpose, a multiplex polymerase chain reaction for the direct detection of FPV and REV was carried out. The obtained results revealed that REV was present in chicken and turkey strains of poxviruses, while the pigeon isolate was negative.
The source of REV contamination is not certain. It is not known whether the vaccine strain used in Croatia was contaminated or REV originated from natural infection. The immunosuppression caused by REV is believed to be responsible for high mortality seen in chicken and turkey flocks.

STRESS INDUCTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 PROTEIN R (VPR) ON FISSION YEAST

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Vpr is a regulatory protein of HIV-1. It plays a dominant role in the pathogenesis of this virus. Several researches show that oxidative stress in human cells is inducted by Vpr. Human cells infected by HIV-1 show oxidative stress phenomena, with different levels of glutathione, superoxid-dismutase in infected patients. To investigate this effect, we used fission yeast cells with the single integrated form of two different types of Vpr. NL4-3 and F34I with an inducible nmt1 (no message in thiamine) promoter were studied. The NL4-3 causes morphological changes in cells and leads to apoptosis. The cells will be elongated in F34I mutant Vpr expression showing G2/M cell cycle arrest, but never

leads to apoptosis. The presence of the viral protein can be detected from 16 hours after gene induction in cells, and by 48 hours it caused apoptosis. During our research, in a cell's life cycle the Vpr induced stress responses were analysed at the 14 and the 35 hours time points according to the doubling times of the strains. Enzyme activities, such as that of peroxidase, superoxidase, GSH, GSSG, catalase, glutathione reductases, were measured.
Different levels of enzyme activities were measured, and there was a significant difference in the peroxide concentration of the two strains containing different types of Vpr. The superoxide production of cell cultures at 35 hours was much lower than at 14 hours in each studied strains, and a difference was detected between thiamine treated and untreated strains.

MURID HERPESVIRUS 4 (MUHV 4) AS ANIMAL MODEL FOR HUMAN LYMPHOTROPIC GAMMAHERPESVIRUS INFECTIONS

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MuHV 4 is a natural pathogen, originally isolated from free-living rodents in the former Czechoslovakia. At least 6 antigenically closely related MuHV strains (MHV-60, -68, -72, -76,-78 and MHV-Šumava) were described. From these, MHV-68 has been studied worldwide. Based on the homology of its double stranded DNA, MuHV was classified as a member of the *Gammaherpesvirinae* subfamily, genus Rhadinovirus. Following intranasal inoculation of mice, MuHV infects lung alveolar epithelial cells, macrophages and B lymphocytes, which carry the virus to mediastinal lymph nodes, spleen and bone marrow. An infectious mononucleosis (IM) like syndrome develops during the acute phase of infection, when atypical T/CD8 lymphocytes eliminate B cells expressing the viral proteins. Similarly to other gammaherpesviruses, MuHV establishes a life long latency especially in B lymphocytes. Productive virus replication is turned on by virus coded *Rta* protein, expression of which is down-regulated by the host cell transcription regulator NFkB. During non-productive latency, a number of latency associated proteins can be expressed, such as M2, M3 (a chemokine binding protein), M11/v-*Bcl-2* (anti-apoptotic protein), ORF72/v-Cyclin (a cellular D cyclin analog), ORF73/LANA (homologous to EBNA1 and to the latency associated nuclear antigen 1 encoded by Kaposi´s sarcoma herpesvirus), and ORF74/v-GPCR (a viral G-protein coupled receptor, homologous to the IL-8R, which activates the *Ras/*c-Jun/AP1 signaling). Properties of the above mentioned non-structural proteins clearly indicate that their expression not only helps to maintain the latent genome during division of carrier lymphocytes, but also induces immortalization. Indeed, a lymphoproliferative syndrome occurs in a proportion of mice at late post-infection intervals; in addition, a leukemia like syndrome or solid tumors may develop. This lecture summarizes our recent knowledge on murine herpesvirus pathogenesis, when stressing the mechanisms of immune evasion, the molecular basis for establishment of latency and at induction of lymphoproliferation. It also highlights the controversial opinions concerning this animal model showing several features of the EBV and KSHV infections.

TRANSFORMING GROWTH FACTOR-BETA 1 (TGFß-1) STIMULATES THE EXPRESSION OF THE EPSTEIN-BARR VIRUS BZLF1 IMMEDIATE EARLY GENE PRODUCT ZEBRA BY MECHANISMS WHICH REQUIRE THE ERK1,2 MAPK AND TWO NF-KB PATHWAYS

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The Epstein-Barr virus (EBV) is a ubiquitous human herpes virus, which is associated with several tumors. EBV latently infects its target cells. Viral reactivation leads to an increased susceptibility to develop an EBV+ lymphoma in immunocompromised hosts. ZEBRA, the viral protein product of the immediate-early EBV gene, BZLF1, mediates the disruption of EBV latency. The physiological stimuli responsible for viral reactivation are not well characterized. We have shown that transforming growth factor-beta 1 (TGF-ß1) triggers the reactivation of EBV in Burkitt's lymphoma cell lines (BLL) and in lymphoblastoid cell lines (LCL): TGF-ß1 induces a time dependent activation of BZLF1 transcription with a corresponding increase in the production of the ZEBRA protein.
TGF-ß1 has been shown to exert its effects through a wide range of intracellular routes, the TGF-ß1 ligand induces the formation of a complex between type I (termed ALK) and type II TGF-ß receptors, which in turn phosphorylates receptor-activated Smads. Interaction with co-Smads provokes their translocation to the nucleus where they bind to Smad-binding elements. TGF-ß1 can also activate alternative signaling pathways including that involving MAPK.
We have explored the signaling pathways mediating ZEBRA expression upon TGF-ß1 induction in different BLL. Our results show that the early events of TGF-ß1 signaling may be mediated by ALKI and ALK5 receptors, since both Smads1 and 2 phosphorylation (which are mediated by ALKI and ALK5, respectively) occurred shortly after TGF-ß1 addition. Furthermore, two different inhibitors of NF-kB and two different inhibitors of ERK1,2 abolish ZEBRA expression induced by TGF-ß1, suggesting that both NF-kB and ERK1,2 activities are required for ZEBRA expression through TGF-ß1. We observed that NF-kBp65 is transiently activated through TGF-ß1 incubation followed by ERK1,2 phosphorylation. NF-kBp65 may participate in ERK1,2 phosphorylation, since the use of an IKK2 inhibitor (that prevents the induction of NF-kBp65 translocation) inhibits not only ZEBRA expression but also ERK1,2 phosphorylation. ERK1,2 phosphorylation is then followed by NF-kBp52 activation. This last step is ERK1,2 independent. However, upon incubation with the IKK2 inhibitor, activation of NF-kBp52 is inhibited, suggesting that activation of NF-kBp52 may be NF-kBp65 dependent. In conclusion, as both activations of ERK1,2 and NF-kB provide protection against apoptosis, our results suggest that TGF-ß1 gives signals for cell survival, which are required for EBV reactivation. We then propose a model, in which the timing and the strength of TGF-ß1 stimulus may influence the final output. TGF-ß1 may induce an early transient increment of NF-kB and ERK1,2 activities, which produce a transient anti-apoptotic effect, which may be followed by apoptosis in the late step of the EBV lytic cycle. The possible impact on cell transformation is discussed.

TRANSCRIPTIONAL ANALYSIS OF GENES ENCODING PROTEINS INVOLVED IN THE BIODEGRADATION OF SULFANILIC ACID

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A bacterial strain identified as *Sphingomonas subartica* SA1 was isolated in our lab. A unique feature of this strain is that it was solely able to use sulfanic acid as exclusive carbon, nitrogen and sulfur source under aerob conditions. Sulfanilic acid is used in big quantities as an intermedier compound in the dye and pharmaceutical industries, although it is not environmentally friendly. Our isolate can also utilize other aromatic compounds such as sulfocatechol, protocatechol, para-aminobenzoic acid, 3,5-dihydroxy-benzoic acid. Investigation on the protein patterns of cells grown on different substrates revealed that alternative metabolic pathways were used for decomposition of the various sub-

strates. However, a similar pattern appearing upon sulfanilic acid and sulfocatechol induction suggested that the two substrates were metabolized via the same pathway and sulfocatechol might be the first intermedier deriving from the oxidation of sulfanilic acid.
The proteomics approach was used to identify the components of the sulfanilic acid degradation pathway. The following proteins specifically induced by sulfanilic acid were identified by mass spectrometry: sulfocathecol-dioxygenase (*scdEF*), a putative glutamine synthase (*glnA*) and a highly conserved but hypothetical oxidoreductase (*scaD*). The genes encoding for these proteins were identified in two genomic locuses. In these locuses, several genes coding for proteins that likely play a role in the degradation of sulfanic acid were also recognized: for example the genes of sulfomuconate cycloisomerase (*scaE*), sulfolactone hydrolase (*scaB*) and maleilacetate reductase (*scaC*) were identified upstream from the *scaA* gene. These enzymes, together with the sulfocatechol dioxygenase, were overproduced in *E. coli* and it was proved that this set of enzymes was enough to convert sulfocatechol to β-ketoadipate.
The genomic context of the identified genes suggests that their protein products participate in the same metabolic pathway. However, the disclosure of their transcriptional organization and regulation would provide further evidence for the functional linkage of the proper gene products.
Reverse transcription coupled PCR was performed to prove that the respective genes were located on the same transcript. Real-time PCR was employed to study the changes in the gene expression levels in cells grown on various substrates. Furthermore, a gene, named as *pcaR,* was also recognized, which likely encodes a LysR type regulator protein, presumably involved in the transcriptional control of the genes of the sulfanilic acid pathway. Its functional analysis is in progress.

MOLECULAR DETECTION OF HEPATITIS E VIRUS (HEV) IN NON-IMPORTED HEPATITIS CASE - IDENTIFICATION OF A POTENTIAL NEW HUMAN HEPATITIS E VIRUS STRAIN IN HUNGARY

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Hepatitis E virus (HEV) is one of the most common causes of hepatitis in endemic areas. However, it has recently been demonstrated that human infection may occur in developed countries without any travel history and that swine may act as a reservoir. The objective of this study was to identify hepatitis E virus by molecular methods in patients with acute non-A-C hepatitis infection with no recent travel history in Hungary and to determine the viral genetic relationship to known HEV strains. Laboratory diagnosis of hepatitis E virus infection was performed in patient sera by HEV IgM and IgG ELISA, IgM and IgG immunoblot and reverse transcription polymerase chain reaction (RT-PCR), using primers for partial viral capsid region. A patient with acute hepatitis with unknown origin was treated at the Hospital of Szeged in June 2004. The patient's acute serum was positive by HEV IgM and IgG confirmed by immunoblots. Viral genome was successfully amplified in the serum by RT-PCR. By sequence- and phylogenetic analysis the virus, designated Hungary1, showed 95% nucleotide identity to genotype 3 hepatitis E viruses related with highest identity to a swine HEV strain (p354/1/02) and having 90% nucleic acid identity to human strain (Greece2). Hepatitis E virus infection is already present in Hungary, without the need to travel to known endemic regions. The first identified HEV in Hungary, which represents a new human genetic lineage, supports the possibility of the endemic infections caused by genotype 3 strains in developed countries and that swine may act as reservoir of human HEV.

COCIRCULATION OF GENOTYPE IA AND NEW VARIANT IB HEPATITIS A VIRUS (HAV) STRAINS IN OUTBREAKS OF ACUTE HEPATITIS IN HUNGARY - 2003/2004

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Hepatitis A virus (HAV) is the most important cause of acute infectious hepatitis worldwide. In Hungary, the reported number of HAV infections has been decreasing in the last four decades, however, every year approximately 500-800 new cases and recurrent outbreaks occur, particularly in the North Eastern region of Hungary. Serology is a routinely used method for laboratory diagnosis of HAV infections, although there was no molecular detection and genetic analysis of the circulating outbreak strains of HAV in Hungary. Serum samples from symptomatic patients were tested from three acute hepatitis outbreaks (OB1 - from low prevalence region in South Western Hungary in 2003 and OB2 and OB3 from the endemic region in North Eastern Hungary in 2004) by enzyme-immunoassay (EIA) and reverse transcription-polymerase chain reaction (RT-PCR) based on 359 bp viral VP1/2A region. By sequence and phylogenetic analysis all HAV sequences belonged to genotype I. OB1 caused by the new variant subtype IB, OB2 and OB3 caused by genetically identical subtype IA strains. The Hungarian IA and IB hepatitis A viruses had the highest nucleotide identity, 99.0% and 98.4%, to IT-MAR-02 and IT-SCH-00 strains detected in year 2002 and 2000 in Italy, respectively. Cocirculation of subtype IA in endemic regions and probably imported new variant subtype IB HAV viruses in non-endemic regions were detected in hepatitis outbreaks in Hungary. HAV strains in Hungary are genetically closely related to strains in the Mediterranean area, especially Italy.

EPIDEMIC SPREAD OF RECOMBINANT NOROVIRUSES WITH FOUR CAPSID TYPES IN HUNGARY

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Noroviruses are the predominant etiological agents in Hungary and common pathogens worldwide in outbreaks of gastro-enteritis in humans. Noroviruses are genetically a diverse group of viruses with multiple genogroups (GG) and genotypes. More recently, naturally occurring recombinant noroviruses were identified. These viruses had a distinct polymerase gene sequence (designated GGIIb/Hilversum) and were disseminated through waterborne and food-borne transmission in Europe, including Hungary. Our aim was to characterize these emerging recombinant noroviruses causing outbreaks of gastro-enteritis in Hungary. From January 2001 to May 2004, samples containing “GGIIb/Hilversum polymerase” (GGIIb-pol) were selected for analysis of the viral capsid region (ORF2) by reverse transcription-polymerase chain reaction (RT-PCR) and sequencing. Thirty-four (14.4%) of 236 confirmed norovirus outbreaks were caused by the variant lineage with the GGIIb-pol. Four different recombinants were detected with capsids of Hu/NLV/GGII-3/Mexico/1989 (n=9, 43%), Hu/NLV/GGII-2/Snow Mountain/1976 (n=6, 28%), Hu/NLV-1/GGII/Hawaii/1971 (n=4, 19%)

and Hu/NLV/GGII-4/Lordsdale/1993 (n=1, 5%). Interestingly, outbreaks caused by recombinant GGIIb-pol strains were mostly associated with outbreaks among children (47%) and had a distinct non-winter seasonality. In Hungary, emerging recombinant noroviruses became the second most common norovirus variants – next to the endemic GGII-4/Lordsdale virus - causing epidemics of gastroenteritis in the last four years.

PRODUCTION AND PURIFICATION OF ALPHA-GALACTOSIDASES FROM *THERMOMYCES LANUGINOSUS*

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α-galactosidases (α-D-galactoside galactohydrolase, EC 3.2.1.22) are spread widely in nature. They can be found in plant and animal tissues, moreover several microorganisms synthesise them. The activity of α-galactosidases may be exploited for different purposes such as: to enhance the yield of sucrose by the hydrolysis of raffinose in the beat sugar industry, to reduce or eliminate the flatulence induced by α-galacto-oligosaccharides of legume based foodstuffs; to modify the galactose content of different galactomannans and thereby may change their technological characters, moreover it can be applied for analytical purposes in the determination of blood-type and in the exploration of structure in the field of glycobiology.

Several fungal α-galactosidases have been studied by different authors and based on the results reported so far, these enzymes differ in their physicochemical and kinetic properties. Until now, limited information was available about the α-galactosidases of thermophilic fungi.

The objective of this study was to reveal the extracellular α-galactosidase production of a thermophilic fungus, *Thermomyces lanuginosus*. Several artificial and natural substrates were applied for induction of the enzyme synthesis. α-Galactosidase activity was observed, when sucrose, raffinose, lactosucrose, L-arabinose and galactomannans were used as sole carbon sources. The production of the α-galactosidase enzyme was also induced in the presence of wheat bran and pea flour.

The best enzyme activity was reached on sucrose, applying *Thermomyces lanuginosus* CBS 395.62 strain. Considerable activity was observed on galactomannans as growth substrates. Comparing galactomannans from different origins, locust bean gum (LBG) was a better inducer for α-galactosidase than guar gum (GG). The enzyme production was optimised in both sucrose and LBG containing media using shaken flask fermentation. The α-galactosidase activity on sucrose was about 100 U/mL after seven-day fermentation, while on LBG this value was less by one order of magnitude.

The enzymes were isolated and purified from the filtrate of the ferment broth. By the developed procedure 42 percentage of the enzyme activity was recovered with a 124-fold purification in the case of the culture medium containing sucrose. When the fungus was grown on medium containing LBG, 22 percentage of the enzyme activity was recovered with about a 900-fold purification. The homogeneity and molecular masses of the enzyme preparations were proved and determined by SDS-PAGE. The molecular mass of the enzyme produced on sucrose and LBG were estimated to be 94 kDa and 53-54 kDa, respectively. These results suggest that *T. lanuginosus* produces at least two different a-galactosidase enzymes.

This observation coincides with the data available in the literature that the fungal α-galactosidases can be divided into different classes based on their characteristics. Some of these α-galactosidases have molecular masses of 45 to 56 kDa, whereas others are in the range of 70 to 95 kDa.

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IMMUNE PROTECTION AGAINST PORCINE PARVOVIRUS DISEASE BY AN IMMUNE COMPLEX-BASED VACCINE

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An insoluble immune complex (IC), prepared under condition of double diffusion in gel using the suspension of the ultrasonic treated PK-15 cell line infected with porcine parvovirus (PPV) and anti-PPV polyclonal immune sera, was used to vaccinated pigs against PPV. SDS-PAGE and Western blot analyses showed that IC contained solely VP2 protein of PPV as an antigen (Ag) and specific antibodies (Abs) of the IgG class. The IC, prepared in the form of a water-in-oil-in-water emulsion (WOWE), was administered intramuscularly. Gilts were immunized with 1 ml IC-vaccine, containing 0.03 ml of viral antigen, 6 weeks before mating and revaccinated after 2 weeks. The protective potency of IC-vaccine was compared with that of a commercially available, inactivated oil-vaccine. The IC vaccination resulted in the development of high and long-lasting anti-PPV Ab titers, similar to that generated by the licensed vaccine ($P > 0.01$), in spite of the fact that the content of virus material administered by IC was more than half than in the licensed vaccine. IC-based vaccines belong to non-replicating, subunit vaccines and represent ecologically convenient and the safest vaccines.

ADHESION AND INVASION PROPERTIES OF *CAMPYLOBACTER JEJUNI* IN CACO-2 CELLS

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Campylobacter is currently recognized as the leading cause of bacterial gastroenteritis worldwide. The species of *Campylobacter*, which are responsible for food poisoning, are classified as thermophilic, growing at 42°C but not at 25°C, microaerophilic, requiring low oxygen content for growth. However, during transmission through the contamination cycle, *Campylobacter* is constantly exposed to stressful conditions such as changes in temperature, nutrient availability and oxygen saturation. In an in vitro model using Caco-2 cells, the cell adherence and invasion abilities of two *Campylobacter jejuni* isolates (poultry and of clinical origin) were studied. Additionally, the influence of environmental stress on adhesion and invasion properties was investigated.

The present study showed that both *C. jejuni* strains adhered to and invaded Caco-2 cells. The human clinical isolate appeared to be more invasive than the animal isolate, but the difference did not reach the required level of statistical significance. The binding and invasion of *C. jejuni* in Caco-2 cells seemed to be enhanced by 5 hour exposure to atmospheric oxygen, especially when the human isolate was used. The growth pattern of oxygen stressed *C. jejuni* was characterized by a rapid intracellular replication in Caco-2 cells, reaching maximal values at 72h post infection. At the completion of the assay (5 days), a significant population of cells still remained viable, as confirmed by cultivation on blood agar plates. The consequence of *C. jejuni* exposure to environmental stress and subsequent changes in the physiological state and virulence properties of the bacteria are discussed.

A HIGH PERCENTAGE OF THE HUNGARIAN HEALTH CARE WORKERS IS NOT PROTECTED AGAINST HAV

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The aim of this study was to evaluate the prevalence of anti-HAV antibody in hospital staff in selected Hungarian institutions. Cross-sectional study was carried out by testing 2132 sera taken from health care workers for the presence of the serological marker of hepatitis A virus. The results were compared to those of different age groups of a seroepidemiological survey carried out in 2000 in Hungary. The anti-HAV seroprevalence was found to be significantly lower in the cohorts of the health care workers above 40 years of age than that in the average population. An increased immunisation activity seems necessary among hospital staff.

DIVERSITY OF REED (*PHRAGMITES AUSTRALIS*) STEM BIOFILM BACTERIAL COMMUNITIES IN TWO HUNGARIAN SODA LAKES

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The common reed, *Phragmites australis* (Cav.) Trin ex Steudel is a cosmopolitan, highly resilient and very productive emergent macrophyte. Ecosystems showing the biggest *Phragmites*-dominance in Europe can be found, for example: in the delta of the River Danube, in Lake Fertő, situated at the Austrian and Hungarian border and in Lake Velencei, the third biggest lake of Hungary. Reeds can also be found at soda lakes of Duna-Tisza köze and Hortobágy (Hungary). The aim of this study was to get insight into the species composition and activity of reed stem biofilms in Kelemen-szék (a soda lake situated in Kiskunság National Park) and Nagy-Vadas (Hortobágy National Park) using traditional cultivation-based and cultivation-independent molecular methods.
Reed stem samples were excised at 10-20 cm below the water surface. Approximately 100 cm^2 of reed stem biofilm was washed with a sterile toothbrush into 200 ml physiological saline solution. This biofilm homogenate was used for the different investigations. Bacterial isolates were gained after serial dilutions and plating onto four different media. After a primary selection 164 bacterial strains were investigated by "traditional" phenotypic tests. The results showed that the 164 strains cultivated from the reed biofilm samples of Kelemen-szék and Nagy-Vadas were fairly inactive in the biochemical tests. Carbohydrate and biopolymer decomposing activities were low in both samples. The bacterial strains of Kelemen-szék showed higher activities in almost all of the tests, except for hydrolysis of gelatin (48% vs. 62% of Nagy-Vadas strains).
Strains were clustered by numerical analysis on the basis of the test results. To identify the taxa, the phenotypically identical representatives were subjected to 16S rDNA-based grouping (ARDRA). The already genotypically and phenotypically identical group-representatives are being sequenced.
The diversity of the biofilm samples was also investigated by cloning. Screening of the 140 bacterial clones resulted in 45 ARDRA-groups. Sequence analysis of the representatives revealed a great diversity. A considerable majority of the clones were affiliated with uncultured bacterial clones (with sequence similarity between 93 and 99%) originating from diverse environmental samples (for example salt marshes, compost or wastewater treatment plants). The DNA sequences of other clones

showed the presence of the genera *Flavobacterium*, *Sphingobacterium*, *Pseudomonas*, *Rheinheimera* and *Agrobacterium*. Based on the results of sequencing the 16S rDNA of the representative clones, a greater metabolic diversity can be hypothesised.
In conclusion, reed biofilm bacteria may be able to adapt to the fluctuating aerobic-anaerobic microenvironments by varying their metabolism from aerobic oxidative to anaerobic oxidative or fermentative. Partly due to their metabolic diversity, partly to their plant-growth promoting or even their plant pathogenic characteristics, these microbes fulfil diverse roles in the biofilm on reed stems.
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COMPARISON OF DIFFERENT PCR METHODS FOR PAPILLOMAVIRUS DETECTION

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Human papillomaviruses (HPVs) belong to the family Papovaviridae. HPVs are strictly species specific and epitheliotropic; they infect epithelial cells either of the skin or the anogenital and oropharyngeal mucosa. Until now, 130 HPV types have been identified and fully sequenced. Approximately 40 types infect the anogenital tract and a few types are commonly found in specimens of anogenital cancer biopsy,, notably cervical cancer. Detection and typing is useful and important for the diagnosis of HPV associated diseases, notably cervical precancerous lesions and cervical cancer. The molecular methods used for HPV testing are based on the method of hybridization, DNA amplification (polymerase chain reaction – PCR) or both. The PCR is, actually, the most specific and the most sensitive method for revealing the presence of otherwise undetectable quantities of HPV DNA. The method allows the detection of a wide spectrum of HPVs using general (consensus) primers. The identification of HPV types may be performed either by hybridization with type-specific probes, or by PCR using type-specific primers.
In this study, 100 DNA samples, isolated from cervical scrapes with abnormal cytology, were analyzed for the presence of HPV by PCR. Five pairs of type specific (TS) primers for HPV 6/11, 16, 18, 31 and 33 (Husnjak et al., J Virol Methods, 2000) and 4 sets of consensus primers: MY09-MY11 (Manos et al., Cancer Cells, 1989), L1C1/L1C2-1/L1C2-2 (Yoshikawa et al., Int. J. Cancer, 1990), PGMY09-PGMY11 (Gravitt et al., J. Clin microbial, 2000) and LCR-E7 (Sasagawa et al. 2000) were used. The most widely used consensus primers are MY09-MY11 primers that are degenerate primers, while PGMY09-PGMY11 consists of a set of 5 forward and 13 reverse primers located in the same region of the HPV L1 gene. Both are generating amplicons of approximately 450 bp depending on the HPV type. L1C1/L1C2-1/L1C2-2 primers consist of one forward and two reverse primers also located in the HPV L1 gene upstream of the MY09-MY11 primers and generating amplicons of approximately 250 bp depending on the HPV type. LCR-E7 primers consist of 4 forward degenerate primers located in the LCR region and 4 reverse primers located in the E7 region generating amplicons of 600 to 758 bp depending on the HPV type.
There were 50 negative and 50 positive TS PCR samples. Each sample was tested by 4 sets of consensus primers, MY09-MY11, PGMY09-PGMY11, L1C1/L1C2-1/L1C2-2 and LCR-E7. Our preliminary findings indicate that the MY09-MY11 and the LCR-E7 primer sets had similar sensitivity but both were less sensitive than L1C1/L1C2-1/L1C2-2 and PGMY09-PGMY11. In conclusion, in order to detect the wide range of HPV types by PCR in a clinical sample, it is necessary to use the most sensitive and specific consensus primers. In clinical practice, beside consensus primer directed

PCR for HPV DNA screening, type specific primer directed PCR should be used for the detection of the most common oncogenic HPV types in a certain population, that is HPV 16 in Europe.

IDENTIFICATION AND CHARACTERIZATION OF *PSEUDOMONAS* STRAINS ISOLATED FROM DEFORMED FRUIT BODIES OF *PLEUROTUS OSTREATUS*

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Pleurotus ostreatus is one of the most extensively cultivated mushrooms in the world, but significant losses in crop and quality arise from blotch diseases caused by different bacterial pathogens. Brown blotch disease, caused by *Pseudomonas tolaasii* is well known, but other fluorescent *Pseudomonas* strains, interacting with *P. tolaasii* can cause light brown coloured symptoms as well. In this study, the Pseudomonads of an infected *Pleurotus ostreatus* farm in Hungary were investigated.
Sixty strains, belonging either to the fluorescent or to the non-fluorescent groups, were isolated on *Pseudomonas* selective S-1 medium, from infected mushrooms and water samples. The biochemical characterization of the isolates revealed that 20% of the strains had gelatin hydrolase, 80% had caseinase, and 60% had lipase activity. All the strains with gelatinase activity had caseinase activity as well. The molecular characterization of 46 strains was performed by PCR based methods. The toxin tolaasin and its derivatives, secreted by the pathogenic form of *P. tolaasii* to disrupt the cellular membrane by forming pores, are non-ribosomal lipodepsipeptides synthesized by a multi-enzyme complex. The PCR reaction with the *P. tolaasii* specific primers, designed from the nucleotide sequence of DNA involved in tolaasin production, revealed that only 2 out of the 46 isolates belonged to *P. tolaasii*, suggesting that other pathogenic *Pseudomonas* species were present in the infected samples. To place these isolates into groups, their genotypic diversity was evaluated by REP and BOX-PCR fingerprints. Based on these results, 39 different patterns were identified, however, 7 out of 39 were similar. To reduce the number of possible groups, further biochemical and molecular investigations are under way and finally the identification of each group will be performed by the sequence analysis of the 16S RNA region.
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GENETIC CHARACTERIZATION OF GRAPE-INFECTING *BOTRYTIS CINEREA* POPULATIONS FROM THE EGER WINE REGION, HUNGARY

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Botrytis cinerea (teleomorph: *Botryotinia fuckeliana*) is a phytopathogen fungus that causes grey mould on a wide range of plants in temperate regions worldwide. *B. cinerea* has been shown to have several variable genetical and physiological traits, and it has developed resistance against most of the fungicides used to control it.

Modern phytopathology is increasingly taking into account the genetic structure of pathogen populations in order to gain insight into control strategies. In light of recent findings concerning *B. cinerea*, it appears that a major cause of the difficulties in managing plant disease indeed arises from our limited understanding of the genetic structure of *B. cinerea* populations. The complexity and variability of this fungus makes it difficult to control and may actually reflect the existence of several distinct populations of which we were unaware, and which may have different characteristics.
Our aim was to evaluate the genetic diversity of *B. cinerea* in the Eger wine region and to determine whether the three genetically different groups *transposa*, *vacuma* and *boty*, earlier described in France and Chile, were present in this region. *Transposa*, *vacuma* isolates were found and, in addition, isolates containing *Flipper* alone (*flipper* isolates) were also detected. Sequence analysis of MSB1 minisatellite and *tef1* (translation elongation factor 1) revealed a high degree of genetic diversity, with no widespread clonal lineages.
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THE EFFECT OF HERBICIDES USED IN MAIZE CULTURES ON SOIL MICROBES AND THEIR ACTIVITY

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Modern crop production is unthinkable without the use of herbicides, but their improper use for several years can greatly reduce soil fertility and can have a disadvantageous effect on the microbiological processes of the soil, which have an important role in the transformation of materials and in the energy flow. In the small-plot experiment set up at the experimental site of the University of Debrecen Department of Plant Protection, the effects of four herbicides, used in maize cultures (Acenit 880 EC, Frontier 900 EC, Merlin Sc, Wing EC), on soil microbiology were studied. The herbicides were applied in increasing dosages (one, two and five times the suggested dosage per ha) to maize and the effects on the microbial activity of the soil were recorded. The herbicides were selected based on previous in vitro studies and on their importance in maize production. The applied herbicides belonged to the categories of “weak poison” and “practically not poisonous”. During the experiment, soil samples from the 2-20 cm soil layer were collected several times. For determining the number of soil microbes, the total number of bacteria (on meat soup agar) and the number of microscopic fungi (on peptose-glucose agar) were determined by plate dilution from an aqueous soil suspension, while the number of nitrifying and cellulose-decomposing bacteria was calculated according to Pochon by the “most probable number of germs” method. The amount of CO_2 released by the soil was measured and also the carbon and nitrate content and microbial biomass were determined by fumigation-extraction method. The nitrate content of the soil and the degree of nitrate mobilization were also determined.
Our results can be summarized as follows. The total germ count measured in the treatments was generally lower than that of the control. The number of microbes decreased considerably with increasing herbicide dosages. At the second sampling, a considerably lower number of microbes was detected. The number of microscopic fungi in the first sampling, except for Frontier, decreased with increasing herbicide concentration. At the second sampling, the number of fungi in the treatments was also smaller than that of the control. In the beginning, the number of nitrifying bacteria increased due to the applied herbicides, except for treatments with Wing. In later examinations, an inhibitory effect was observed. The treatments had basically no influence on the occurrence of cellulose-decomposing bacteria. The amount of carbon in the microbial biomass decreased considerably after the treatments

as compared to the control, except for treatments with Frontier. At subsequent samplings, a decreasing difference was measured. A similar trend was observed for nitrogen in the microbial biomass. At the first sampling, an increase was detected in soil respiration (CO_2 production) as a result of the treatments. Later, the differences between the treatments levelled off. When evaluating the nitrate mobilization in the soil, no differences were measured between the treatments. At subsequent samplings, the measured values were always higher than those of the control and increased with increasing dosages, except for treatments with Merlin.

INTRACELLULAR TRAFFICKING OF *LEGIONELLA PNEUMOPHILA*

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Legionella pneumophila is an intracellular pathogen that modulates biogenesis of its phagosome to evade endocytic vesicle traffic. The *Legionella*-containing phagosome (LCP) does not acquire any endocytic markers and is remodeled by the endoplasmic reticulum (ER) during early stages. We show that intracellular replication of *L. pneumophila* is inhibited in IFN-g activated bone marrow-derived mice macrophages and IFN-g activated human monocyte-derived macrophages (hMDM), in a dose-dependent manner. This inhibition of intracellular replication is associated with maturation of the LCP into a phagolysosome, as documented by the acquisition of Lamp-2, Cathepsin D, and the lysosomal tracer Texas Red Ovalbumin, and with the failure of the LCPs to be remodeled by the RER. We conclude that IFN-g activated macrophages override the ability of *L. pneumophila* to evade endocytic fusion, and the LCP is processed through "default" endosomal lysosomal degradation pathway.

HIGHLY VIRULENT INFECTIOUS BURSAL DISEASE IN BROILER CHICKENS WITH NO OR LOW MORTALITY BUT WITH REDUCED BODY MASSES AND IMMUNOSUPPRESSION

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Highly virulent infectious bursal disease (IBD) viruses, in contrast to classical and variant IBD viruses, cause usually 10% mortality of susceptible broiler chickens but sometimes it may reach 25%.
An outbreak of IBD in vaccinated broiler chickens in Croatia is generally described with no increased mortality but significantly reduced body weight gain. Experimental infection of fully susceptible 30-day-old broilers with IBD virus isolated from affected chickens resulted in no mortality and mild clinical signs, but body weight gains were significantly reduced (52.9 grams and 83.6 grams per day in susceptible and immune birds, respectively) during the two weeks following contact infection. Rapid seroconversion was noted in unvaccinated birds. Induced transcription of interferon alpha and gamma of susceptible broilers was significantly reduced on the 7th and 14th day after exposure to the isolated virus, whereas induction in broilers vaccinated against IBDV was not reduced. Infection of 4-week-old Leghorn type SPF chickens with the virus resulted in severe disease with 60% mortality, which implicated that the isolated IBD virus was highly virulent although it did not cause high mor-

tality of susceptible broiler chickens. Nucleotide sequencing of the hypervariable region of the VP2 gene confirmed that the virus was genetically similar to highly virulent IBD viruses.

GENETIC BACKGROUND OF THE DIFFERENCES IN THE SPECIES SPECIFICITY OF BOVINE ADENOVIRUS SUBTYPE A AND SUBTYPE B

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Bovine adenovirus type 2 (BAdV2) may cause asymptomatic infection or mild pneumoenteritis in cattle and/or sheep. Based on the differences in the properties of the genome, the species specificity, the haemagglutination spectrum and the pathogenicity of the strains, the serotype has been divided into two subtypes. The heteroduplexes formed by the nucleic acid of subtype A (prototype strain No19, isolated from cattle) and subtype B (strain ORT/111, isolated exclusively from sheep) were complementary on 95% of the genome. The regions that were not complementary formed two loops located on the E3 and on the fiber regions of the genome. After sequencing about 50% of the genome, including the regions where the smallest identity was presumed, we conclude that the changes in the E3 region (78.5% identity on nucleotide level) and in the fiber knob (89.5% identity on nucleotide and 94.2% on amino acid level) are behind the changes in the species specificity of BAdV2/B. The changes in the hemagglutinating activity (BAdV2/A agglutinates bovine, BAdV2/B rat erythrocytes) most probably are caused by the changes in the fiber knob and shaft. The amino acid identity in the latter region is 56.5% caused by deletions (32 amino acids), insertions (19 amino acids) and substitutions (127 amino acids). The changes detected in the E3 region (deletion of 325 nucleotides, a short insertion, nucleotide changes) may play a role in the delayed immunoreactions of the host organism. The observation that the two subtypes could not be differentiated by virus neutralisation may be explained by the relatively minor changes on the fiber knob and the hexon. The structure of these proteins most probably was not changed significantly enough; therefore the antibodies generated by one subtype could react with the majority of the epitopes of the other subtype. Comparison of the nucleic acid sequences of the two subtypes with the deposited ovine adenovirus 3 (OAdV3) genome revealed that the identity of the BAdV2/B (ovine strain) is higher to the OAdV3 then to the BAdV2/A (bovine strain) in case of certain genes in the E3 region. This higher similarity may be the result of converging evolution in the process of adaptation to the host (sheep), or may be caused by recombination.

The changes on other sequenced parts of the genome were not significant enough to play a role in the changes of the biological properties of the virus.

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THE BLAZING SENSES OF A BIOTECHNOLOGICAL CELL FACTORY: CELLULASE EXPRESSION OF *HYPOCREA JECORINA* IS INFLUENCED BY LIGHT

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Light is one of the most important environmental cues for most living creatures, including humans. Collected light information is transferred to many different biological effectors, and proper perception of ambient light is essential for an organism to adapt to its environment. Studies on differential gene expression in cellulase signal transduction using the *Hypocrea jecorina* (anamorph: *Trichoderma reesei*) wild-type strain QM9414 and the cellulase non-inducible strain QM9978 revealed, that a potential light regulator protein is also involved in this process. This regulator was designated Envoy, and represents a PAS/LOV domain protein with high similarity to the *Neurospora* light regulator VIVID. It links cellulase induction by cellulose to light signalling in *Hypocrea jecorina*: an *env1*$^{PAS-}$ mutant of *H. jecorina* grows significantly slower in the presence of light but remains unaffected in darkness as compared with the wild-type. In contrast, induction of cellulase formation by cellulose is stimulated by light in the wild-type strain, but significantly altered in the *env1*$^{PAS-}$ mutant.
We could show that *env1* encodes a regulator essential for *H. jecorina* light tolerance, connects light response to carbon source signalling and thus suggests the presence of a regulatory network comprising multiple input factors. The complex regulation of *env1,* by light and simultaneously by the type of carbon source, indicates additional factors of influence in experimental design when analyzing regulatory circuits in fungi. Based on these results, we studied the connection of light and cellulase gene expression in more detail and found that not only a protein similar to the *Neurospora* light regulator VIVID, but also the *H. jecorina* homologues interacting with VIVID in *Neurospora* play an important role in cellulase signal transduction. *Trichoderma atroviride* strains lacking the blue light regulator proteins BLR-1 (similar to White Collar-1) or BLR-2 (similar to White Collar-2), respectively, show a strongly increased cellulase production on cellulose. Further investigation of the cellulase signalling network also revealed that the G-alpha protein Tga3 has an impact on cellulase expression, as complementation of the wild-type stain with a constitutively activated version of this protein lead to significantly increased *cbh1*-transcript levels only during cultivation in constant light, thereby again showing the connection to light perception in this fungus.
In search for a factor responsible for this light response, we found an approximately 40 kDa protein being differentially regulated, on the one hand between cultivation in light and darkness on cellulose and on the other hand independent of the light status in a strain deleted for Minute, a small protein, which is regulated by light and depends on the carbon source. Interestingly Envoy is involved in the regulation of Minute most probably by interfering in its autoregulatory feedback loop.

MOBILISATION AND REMOBILISATION OF A COMPLETE PATHOGENICITY ISLAND OF THE UROPATHOGENIC *ESCHERICHIA COLI* STRAIN 536 BY CONJUGAL TRANSFER

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An increasing number of studies on genes and genomes indicate that a considerable portion of horizontal gene transfer (HGT) has occurred between prokaryotes. Lots of virulence associated genes are carried on mobile genetic elements like plasmids, pathogenicity islands (PAIs) and bacteriophages. In our model organism of uropathogenic *Escherichia coli* strain, 536 several virulence associated genes are located on PAIs. Up to now five PAIs have been thoroughly characterised in this strain. The results show that all of them could be deleted from the core chromosome, and in two cases (PAI II536 and PAI III536), the presence of a circular intermediate has also been detected after deletion. By inserting a special construct into PAI II536 that contained mobility genes (*mob*), a replication of origin

(*oriV*) and a replication of transfer (*oriT*), we could verify that this more than 100 kb long PAI can not only be deleted from the chromosome of strain 536, but can also be transferred into another recipient *E. coli* strain. Analysis of our transconjugants has revealed a transfer of the complete PAI II536. In part of the resulting clones the mobilised PAI has been present as a circular intermediate, while in other derivatives site-specific integration of the mobilised PAI has occurred into the tRNA gene *leuX*. This is the gene that PAI II536 is originally associated with in strain 536. Furthermore, the mobilised PAI II536 could be remobilised into a PAI II536 deletion mutant of the wild type strain 536. Our results demonstrate that transfer of a complete PAI is possible not only by transduction but also by conjugation that helps bacterial evolution in quantum leaps.

COMPARATIVE STUDY ON *PASTEURELLA MULTOCIDA* ISOLATES USING TRADITIONAL AND MOLECULAR DIAGNOSTIC METHODS

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Pasteurella multocida is a common inhabitant of domestic and wild animal populations in Hungary just as all over the word. Although *P. multocida* has long been known to cause diseases in a wide range of animal species, our knowledge is rather limited about this pathogen. There are numerous chronic carriers of this microbe in swine and cattle herds as well as in poultry stocks and rabbit-farms. These animals are reservoirs of infection, which may lead to endemics with the aid of various predisposing factors, manifested in acute illness and fatal cases. Severity of symptoms largely depends on the sensitivity of the individual hosts in the stock. It makes the situation more difficult that *P. multocida* can be transmitted by various animal species.

Data refer to a large range of diversity and complex adaptability within this bacterial species, and brings up the possibility of cross-infection between different host species. Protection against *P. multocida* has not been solved. The failure is partially explained by the antigenic and structural complexity of this pathogen. We should learn more about its antigenic determinants, the patho-mechanism of infection and its relation to the host species. In our study, *P. multocida* strains isolated from poultry and rabbit, representing various geographic origins were used to determine the infection rate of Hungarian stocks and the host diversity in the past decade. Special attention was paid to *P. multocida* strains showing uncertain or unexpected biochemical reactions. BSS test (buffered single substrate) was used to detect acid production of the strains from carbohydrates. For the determination of capsular types, traditional biochemical methods (determining hyaluronidase, heparinase and chondroitinase activities of the strains) and multiplex capsular PCR (polymerase chain reaction) were applied. Multiplex capsular PCR was able to establish capsular types of the strains even if they lost their capsules during artificial maintenance (non-capsulated phenotypes). *P. multocida* specific PCR proved to be useful to categorise strains of doubtful taxonomical classification. These techniques may provide us with useful tools to get a better understanding of the antigenic characteristics and pathogenicity of *P. multocida* strains with different biochemical, serological and genetic features.

REGULATION OF CELLULASES EXPRESSION DURING GROWTH ON LACTOSE IN *HYPOCREA JECORINA*

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The ascomycete *H. jecorina* (anamorph *Trichoderma reesei*) is used industrially to produce cellulolytic and hemicellulolytic enzymes and its strong cellulase promoters are of interest for recombinant protein production by this fungus. Cellulase and hemicellulase formation is induced by several mono- and disaccharides, e.g. sophorose, xylobiose, lactose, D-xylose, L-sorbose. Most are too expensive for industrial fermentation, therefore, the preferred carbon source is lactose (1,4-O-β-D-galactopyranosyl-D-glucose) because it is renewable, economically attractive and soluble. In *H. jecorina* lactose is cleaved extracellularly by ß-galactosidases to D-glucose and D-galactose. While D-glucose enters the gylcolytic pathway, D-galactose can be converted by two different pathways. D-galactose is either phosphorylated by a galactokinase, or by an NADPH-linked reduction mainly catalyzed by D-xylose reductase. We have biochemical and genetic evidence that D-galactose is converted in this novel pathway by other enzymes of the pentose catabolic pathways including L-arabinitol 4-dehydrogenase and xylitol dehydrogenase.
We show that both identified D-galactose catabolic pathways are essential for complete cellulase induction on lactose and discuss the importance of both pathways for inducer formation.

THE EFFECTS OF COPPER, NICKEL AND LEAD CONTAMINANTS ON PHOSPHATASE AND DEHYDROGENASE ENZYME ACTIVITIES OF SOIL WITH DIFFERENT GENETIC TYPES

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Soil protection is one of the basics of sustainable development. Metal pollutions change the quantitative indicators of the biological activity of soil e.g.: enzyme activities. The aim of this study was to present data about the effect of copper, nickel and lead on the phosphatase and dehydrogenase activities of different soils. The effect of metal contamination was examined under human intervention of the soil. This effect was determined by measuring the soil's own microbial activity in a laboratory model-experiment.
This study presents results and conclusions about the soil microflora under metal stress. Three different soil types were used in a laboratory model-experiment. The added metals were in the form of water soluble compounds of copper, nickel and lead. Soils were treated with increasing metal concentrations of 0, 50, 200 and 800 kg/hectare. Phosphatase and dehydrogenase activities of three soils were analysed 0, 7, 14 and 28 days after metal addition. The laboratory model-experiment was carried out in three parallels. Based on the results of measurements, the following phenomena were experienced. Copper and nickel decreased but lead increased the enzyme activity in all cases. Generally, the smallest change of enzymatic activities was measured in the moulded and loamy soils (chernozem), whereas the biggest change of enzymatic activities was measured in the acidic soil (brown forest soil). The dehydrogenase enzyme system (oxido-reductase) is more sensitive than the phosphatase enzyme system (hydrolase) in case of soils with large adsorption capacities. A dose-dependent effect was found in the case of copper and nickel, but not in the case of lead.
These experiments help us establish a model for the natural self-cleaning ability of the soil and follow the efficiency of remediation of metal pollution.
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FORK-HEAD-TYPE TRANSCRIPTION FACTOR SEP1P PLAYS A CENTRAL ROLE IN THE REGULATION OF CELL SEPARATION IN *SCHIZOSACCHAROMYCES POMBE*

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Cell separation is the final event of the cell cycle in unicellular cell-walled organisms, during which the daughter cells physically separate. In the fission yeast *S. pombe*, this is achieved by splitting the central layer (primary septum) of the septum developed during cytokinesis (septation). The cleavage of the primary septum is accompanied by local erosion of the adjacent regions of the mother cell wall. The endo-β-1,3-glucanase encoded by the *eng1+* gene and the endo-α-glucanase encoded by the gene *agn1+* are the major enzymes involved in cell separation. The C2H2 zinc-finger transcription factor Ace2p regulates the expression of both genes. Ace2p controls the activity of numerous additional genes participating in citokinesis and cell separation. The transcription of *ace2+* requires Sep1p, a transcription factor containing a fork-head DNA binding domain. Sep1p is a phosphoprotein produced constitutively during the cell cycle. It does not only regulate *ace2+* transcription, but also interacts with cell cycle regulators, such as *wee1+*, *cdc25+* and *cdc2+*.

CULTIVATION STUDIES ON ENTOMOPATHOGENIC NEMATODE – BACTERIUM SYMBIONT COMPLEXES

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The entomopathogenic nematode (EPN) – entomopathogenic bacterium (EPB) complexes have increasing importance as environment friendly biocontrol agents. The microbial symbionts of nematodes kill the insect larvae by means of their endotoxin production, and their antibiotics save the insect cadavers against other soil microorganisms. Moreover, the primary phase variants of EPB-s function as feed for the nematodes. Several practically important biocomplexes can be effectively cultivated in bioreactors but the growth period to achieve a suitable EPN number is quite long (cc. 500 h). The impeller-stirred draft-tube reactors are considered suitable for larger scale-EPN/EPB production since a high oxygen transfer rate can be achieved inside without the dangerous enhancement of the shear force [1].

The goal of our recent project is to survey the Hungarian EPN/EPB fauna, to establish a strain collection from the potentially useful EPN strains and to elaborate a biological control technique against the grubs of the European cockchafer (*Melolontha melolontha*), the most harmful insect pest of Hungarian horticulture. Our newest results concerning the first two aims were presented last January [2].

The subjects of the present cultivation studies are the members of the biocomplex consisting of *Heterorhabditis downesi* '267' EPN and its EPB, a *Photorhabdus temperata* strain. The complex isolated from soils of the region "Nyíribrony 2/D" was found to be effective against cockchafer grubs. The bacterium cultivation experiments were performed first in shake flasks, in LB and TSY medium. Temperature and oxygen supply were the most important parameters examined. It has been established that the aeration rate required by the cells is about 0.6-0.7 vvm. The temperature range of 20-

22°C has been determined as the optimum from the point of view of the sustainability of the culture. With the application of a 5 dm^3 INEL BR 97 type fermenter for *P. temperata* growth studies, a special fed-batch technique has been elaborated. It has been proved that the required living cell number of the primary phase variant of EPB can be sustained for a long time (460 h).
Based on the common cultivation experiments of the *P. temperate* / *H. downesi* complex, it has been found that LCM is the most suitable standard medium to save the viability of nematodes and to get an optimistic growth rate. In this medium, a considerable percent of the dauer juveniles developed into reproductively mature adult hermaphrodites [1], so the initial number ($10^3/cm^3$) of dauer juveniles increased to $1.5\text{-}2\times10^4/cm^3$ within 12 days. At present, the optimization studies of EPN/EPB production are in progress, with special consideration of the reduction of medium costs.
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PHYTOPLASMAS - PROKARYOTES WITH A MINIMAL GENE SET FOR LIFE AND THEIR PARASITISM

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Phytoplasmas are wall-less prokaryotes present as endocellular parasites in the plant phloem and phloem-feeding insects like leafhoppers (*Cicadomorpha*) and planthoppers (*Fulgoromorpha*). These unique bacteria, belonging to a monophyletic clade within the class *Mollicutes*, have the smallest known self-replicating genome. It consists of only 580-1350 kbp, organized mainly in one circular chromosome and it lacks some genes previously considered to be essential for life. In some phytoplasmas, the existence of extrachromosomal elements has been confirmed.
Every attempt to obtain axenic phytoplasma pure cultures has failed, which impedes most aspects of their research. However, much progress has been done in the last two decades due to the application of various molecular biology-based methods. The most important tools for phytoplasma detection and classification are PCR and RFLP analyses of the highly conserved 16S rRNA gene. Lately, other gene regions have been used for finer phytoplasma distinction in order to study their molecular variability and epidemiology. Even though the complete genome of one *Candidatus* Phytoplasma species is known, the classification based on the combination of 16S rRNA genes and biological properties of the phytoplasma (plant host range, symptoms, insect vector species) remains to be the basis for their classification.
Phytoplasmas cause hundreds of plant diseases worldwide and many of them affect economically important crops. On the other hand, insects that harbor and vector these prokaryotes in nature do not necessarily suffer such consequences. The occurrence of phytoplasmas causing grapevine yellows (GY) disease in Croatia was first recorded in 1997. Severely affected vines mostly showed the presence of phytoplasmas from the ribosomal subgroup 16SrXII-A (stolbur or bois noir) while atypical and milder GY symptoms have been observed in Croatian indigenous vines that were infected with phytoplasmas of ribosomal subgroup 16SrI-B (aster yellows). By studying several genes of phytoplasmas found in Croatian vineyards, their insect vectors and alternative herbaceous hosts (vineyard weeds), we hope to decipher molecular and ecological factors discriminating this pathosystem from similar ones in Europe. Insight into the molecular epidemiology of GY should also enable devising more effective ways to control these phytoplasma-associated diseases.

POSSIBLE ROLES AND FUNCTIONS OF AN ABUNDANT, 5.6 KB SMALL PLASMID OF *BACTEROIDES* SPP.

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Bacteroides belong in the three taxa harbouring of small, often regarded as cryptic plasmids with the highest frequency. Epidemiological and molecular analysis have demonstrated three major classes of such *Bacteroides* plasmids (2.7 kb, 4.2 kb and 5.6 kb), all of which are abundant in both clinical and normal microbiota isolates, though with varying frequency in different parts of the world. Our studies have revealed that 50% of Hungarian clinical *Bacteroides* isolates carry such plasmids and 37% harbour the 5.6 kb type, the corresponding figures for normal microbiota isolates being 39% and 18%, respectively. The relatedness of the members of this plasmid type was confirmed by molecular methods, and a polymorphism was detected in an approximately 1.3 kb region. We were interested in the function of this region and a specific conjugation assay was designed to detect whether this region codes for mobilization, or perhaps replication or other functions. The assay demonstrated that it does not participate in replication or mobilization. Nucleotide sequencing revealed a protein coded in the region that could be secreted as a lipoprotein. The gene has a promoter structure specific for *Bacteroides*, a ribosome-binding site and a terminator structure. These results imply that this 5.6 kb plasmid type is mobilizable and has a function that could not involve basic plasmid functions such as replication and mobilization; accordingly, the plasmid type is not cryptic. Determination of the full nucleotide sequence of this plasmid is in progress in our laboratory, and the possible involvement of this lipoprotein in *Bacteroides* pathogenesis as a low potential virulence factor is discussed.

IDENTIFICATION AND EVALUATION OF HLA-A2 RESTRICTED HHV-6 SPECIFIC T-CELL EPITOPES

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Human herpesvirus type 6 (HHV-6) infections are ubiquitous, infection usually occurs during infancy. Seropositivity in the adult population is estimated to be about 95%. The virus persists following primary infection. Reactivation of the virus is an important concern in immunocompromised patients and may also result in complication during pregnancy.
The result of our previous study suggested that humoral immune response may not prevent the reactivation of HHV-6 infection during pregnancy. This finding drew our attention to the prevalence and presumed protective effect of cellular immunity to HHV-6. The aim of the present study is to identify individual immunogenic HHV-6 peptides as well as to evaluate the cellular immune response by determining the frequency of peptide specific CD8+ T cells. In an attempt to define HHV-6 gB and p100 structural protein-derived HLA-A*0201 restricted epitopes, computer prediction was carried out by combining algorithms predicting HLA binding and proteosomal processing. Peripheral blood mononuclear cells (PBMC) of seropositive blood donors with the HLA-A*0201 haplotype were stimulated in vitro with selected synthetic epitopes. The immune response was assessed by flow cytometric evaluation of the appearance of CD69 activation marker on specific T cells. As a result, we have identified several as yet unknown immunogenic HHV-6 epitopes. FLMNSVLMI (Nş 12), LMNSVLMIY (Nş 13), and ILYVQLQYL (Nş 410) oligopeptides of gB origin induced 0,91

(±0,41) %, 1,15 (±0,33) % and 5,16 (±2,33) % increase in the number of CD69 expressing cell, respectively. Among the oligopeptides of p100 origin DVFDPVHRL (Nº 480) induced a 1,19 (±0,56) % increase. In analysing the response of PBMCs to in vitro stimulation, we observed significant differences between individual donors. Based on this experience, we set out to analyse the HHV-6 specific immune response during pregnancy.

DENITRIFICATION IN EXTREME ENVIRONMENTS

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Denitrification is making use of N oxides as terminal electron acceptors for cellular bioenergetics under anaerobic, microaerobic, and occasionally also aerobic conditions. This process is a crucial branch of the global N cycle, reversing dinitrogen fixation, and is linked to chemolitotrophic, phototrophic, diazotrophic, or organotrophic metabolisms, but generally not to obligate anaerobic life. Discovered more than a century ago and believed to be exclusively a bacterial trait, denitrification has been found and studied in halophilic archaea and bacteria, hyperthermophilic archaea, and also in the mitochondria of fungi.

Mahne and Tiedje [1] put forward a now widely accepted criteria for the identification of denitrifying bacterial strains, suggesting that ‘’true’’ pure culture denitrifiers converted N oxides to N_2O or N_2. However, this situation can be achieved only if all denitrification genes are present and expressed in a single cell,. Zumft [2] proposed a modular concept of denitrification in the environment, incorporating also the community level. Denitrification to N_2O or N_2 results from incomplete and truncated denitrification pathways present in various organisms, expressed under *in- situ* conditions.

Organisms containing complete denitrification pathways were retrieved from the environment among others containing only partial or truncated denitrification pathways. However, complete denitrifiers were reluctant for isolation from some hypersaline, alkaline or acidic environments. Functional properties, community structure and environmental parameters are being explored in cold, hypersaline, alkaline and acidic environments at community level. We are now only beginning to realize the organization and contribution of such peculiar environments to global warming, forcing us to reconsider our current global green-house gas emission estimates. Evolutionarily intriguing questions have been raised regarding function-structure relationship of microbial communities. Models on microbial functions are needed to address the question of significance of horizontal gene transfer for the concept of microbial diversity and for the distribution of functional communities and their activities in the environment [3]. We miss the data to make more informed interpretations about how life is patterned, how environments determine those patterns and whether we can begin to infer functional meaning. Being one of the comparatively well-studied environmental processes, could denitrification serve as the working horse, like “*E.coli*”, of environmental functional genomics?

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DEVELOPING A MATHEMATICAL MODEL FOR THE FISSION YEAST CELL CYCLE

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The most fundamental requirement for life is cell reproduction. Cell cycle is the sequence of events by which a growing cell duplicates all its components and partitions them more-or-less evenly between two daughter cells. The events of the cell cycle of most organisms are ordered into dependent pathways in which the initiation of later events is dependent on the completion of earlier events. For example, chromosome segregation in eukaryotes is dependent on the completion of DNA synthesis. These processes are controlled by a considerably complicated regulatory molecular network called the cell cycle machinery. In the first half of the 20th century, yeasts have become model organisms in different fields of cell biology. Since the late '50s, *Schizosaccharomyces pombe* (also known as fission yeast) has been spotlighted through its favourable physiological features, for example, its symmetrical division enables good synchronisation techniques, which are necessary for cell cycle studies. During the last 40 years, *S. pombe* has become an attractive model organism in all chapters of cell cycle research, as well as in other physiological, genetic and biochemical studies. The full genome of *S. pombe* has been recently sequenced, indicating the importance of this species. The aim of the present study was to test a recently published mathematical model of the fission yeast cell cycle by simulating some mutants that were not involved in former research. It has long been known how the behaviour of fission yeast cells changes if they over-produce either the cdc25 or the wee1 gene. These over-producing mutants either have multiple integrated copies of the corresponding gene, or gene transcription is controlled by a very strong constitutive promoter. Because the reversible phosphorylation and dephosphorylation of the Cdc2 subunit in MPF (promoted by the Wee1 and the Cdc25 proteins, respectively) is a crucial point in the regulation of the fission yeast cell cycle, our mathematical model should properly describe the phenotypes of these over-producing mutants. The main goal of this study was to test the model in this respect, and to examine whether there were any discrepancies between the experiments and the simulations. In case there were, some parameters (or some differential equations) of the model should have been modified in order to decrease these discrepancies below the level of acceptance.

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BACTERIAL SPECIES WITH PHOSPHATASE ACTIVITY AND/OR POLYPHOSPHATE RESERVE IN THREE HUNGARIAN SHALLOW LAKES

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Phosphorous has been known to be the dominant limiting nutrient in many freshwater ecosystems. Heterotrophic bacteria play a key role in phosphate regeneration through mineralization of organic matter and solubilizing insoluble phosphorous compounds of the sediment. Numerous bacteria are able to accumulate large quantities of polyphosphate in metacromatic granules and this phosphorous reserve can be readily used by them when the extracellular phosphate levels are low. The aim of this study was to get information about the species taking part in the phosphorus regeneration and accumulation in the biggest Hungarian lake called Balaton (Keszthely-basin, eutrophic; Siófoki-basin, mesotrophic) and two hypereutrophic soda lakes namely Kelemen-szék and Böddi-szék situated in the Kiskunság National Park (KNP).

Samples were taken from the upper 2-5 cm of the sediment regions. A total of 146 (Balaton) and 160 (KNP) bacterial strains were isolated after serial dilution and plating onto six different media. All strains, showing phosphatase activity and/or accumulated polyphosphate, were investigated with cell morphology and conventional biochemical and physiological tests. More than 75% of the strains from each lake accumulated polyphosphate according to the results of Neisser staining. The phosphatase activity varied between 31-60%. A big number of the selected strains were able to decompose different biopolymers, especially lipase and proteinase activities were widespread. On the basis of the studied 30 phenotypical data, a numerical statistical analysis was carried out using Simple Matching coefficient. On the phenotypical dendrogram, strains with an average 85-90% similarity level formed a cluster. To clarify the taxonomic positions of the isolates representative strains (at least one of each cluster and all individual strains) were chosen for 16S rDNA-based grouping (ARDRA). Phenotypically and genotypically identical representatives were subjected to partial sequencing of 16S rDNA.
Most of the isolates showed the highest sequence similarity with species of genus *Bacillus* (*B. pumilus, B. marisfalvi, B. macroides, B. mycoides*). Other strains were placed into groups of High G+C Gram Positive Bacteria (species of the genus *Arthrobacter*), Gamma Proteobacteria (species of the genera *Aeromonas, Pseudomonas, Reinheimera*).

CHARACTERIZATION OF BACTERIA ISOLATED FROM ACUTE PUERPERAL (ENDO)METRITIS IN DAIRY COWS: EVALUATION OF ANTIBIOTIC SENSITIVITY BY MEANS OF MIC

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Acute puerperal (endo)metritis (APE) is a common bacterial complication of postpartum uterine involution in dairy cows, occurring mainly at the first 2 weeks after calving. Several bacteria can be isolated from the uterus of the affected cases. However, only some of them i.e. *Arcanobacterium pyogenes*, *Escherichia coli* and certain Gram-negative anaerobes (*Fusobacterium necrophorum*; *Bacteroides spp*.) play a significant role in the pathogenesis of this disease.
In this survey, we checked the minimal inhibitory concentration (MIC) of bacteria isolated from large-scale Hungarian dairy herds against oxytetracyclin (OTC) and amoxicillin, as well as some cephalosporins (cephapyrin and cefquinome) just recently applied in therapy of APE. Uterine swabs were collected from Holstein-Friesian cows showing at least the local clinical signs of APE in five herds. Isolation and identification of the bacteria were performed according to the conventional bacteriological techniques. The strains were identified up to species levels using appropriate biochemical tests and Bio-Merieux ATB automatic identifying system. For the determination of MIC, 90 µl amounts from broth containing two-fold concentration increments of antimicrobial agents were added to 96-well microdilution trays. Each well was inoculated with 10 µl bacterial suspension (the inoculum was 1.5 x 10^5 CFU/ ml). The MIC was read as the lowest concentration of the antibiotic at which no growth was recorded.
A total number of 33 swabs were collected and cultured. The main isolated pathogens were as follows: *A. pyogenes* (n=28), *E. coli* (n=30), *F. necrophorum* (n=4), bile-resistant *Bacteroides spp.* (n=9), bile-sensitive *Bacteroides spp.* (n=24), black pigmented *Prevotella and Porphyromonas spp*.

(n=10). High MIC (> 1.0 µg/ml) was measured against OTC at more than half of *E. coli* and at almost all Gram-negative anaerobes (except for the few strains of *F. necrophorum*). Also about 25% of *A. pyogenes* strains were resistant to OTC. Almost all *E. coli* and all *bile-resistant Bacteroides spp*. were completely resistant to amoxicillin (MIC > 1.0 µg/ml and MIC ≥ 4.0 µg/ml, respectively). Other Gram-negative anaerobes showed variable MIC, while the majority of *A. pyogenes* strains possessed relatively high *in vitro* sensitivity (MIC ≤ 1.0 µg/ml) to amoxicillin. All the isolated pathogens, but one *E. coli*, proved to be sensitive (MIC ≤ 1.0 µg/ml) to cefquinome, a 3rd generation cephalosporin derivate. *A. pyogenes* and Gram-negative anaerobes showed 100% sensitivity to cephapyrin, however, 75% of the *E. coli* strains had of MIC ≥ 4.0 µg/ml and the remaining 25% were even around 1.0 µg/ml. We conclude that increasing MIC of OTC and amoxicillin against the main uterine pathogens is remarkable, as compared to our previous findings. This tendency justify new ways in antimicrobial therapy of APE in dairy cows.

OCCURRENCE OF VANCOMYCIN RESISTANCE OF ENTEROCOCCI ISOLATED IN A HUNGARIAN TEACHING HOSPITAL, DEBRECEN

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Enterococcus spp. has recently emerged as a major nosocomial pathogen because of its resistance to multiple antibiotics, including glycopeptides. The frequency of vancomycin-resistant enterococcus (VRE) isolates has increased worldwide following reports in 1988. The aim of the present study was to determine the prevalence of vancomycin resistant enterococci in a teaching hospital of Debrecen. The samples were collected from patients between January 2004. and March 2005. Suspicious colonies were identified to species level using routine criteria Rapid ID 32 Strep system (BioMerieux). The 1766 enterococci isolates were screened on a 6 mg/l vancomycin BHI plate (Oxoid Ltd.) according to the NCCLS. The MIC of vancomycin and teicoplanin was determined by E-test method. Resistance to glycopeptides was confirmed by PCR for detection of vanA, vanB,and vanC genes. Seventy-nine *Enterococcus spp*. has been isolated on the screening medium (4.4 %). The most common sites of isolation were in the descending order: urinary tract (77.5 %), pus (7.5 %) ascites (7.5%) materials from genital tracts (7.5 %). We have found 2 strains carrying the van A genes. Our results show that VRE strains have already emerged in Hajdú-Bihar county. It is also probable that the frequency of infections caused by glycopeptide-resistant enterococci will increase in this area.

ROTAVIRUS RNAEMIA IN FINNISH CHILDREN WITH ACUTE GASTROENTERITIS

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An emerging number of studies report that in rotavirus gastroenteritis the infection is not confined only to the intestines, but rarely the virus can spread, in small quantity, into the circulatory system. Rotavirus was detected from the cerebrospinal fluid, as well as from the blood of infants with rotavi-

rus diarrhea [1-4]. This study aimed to determine and type rotavirus in serum samples of young children hospitalized with acute gastroenteritis in Finland.
Children from 6 to 36 months of age hospitalized due to dehydration caused by acute gastroenteritis at Tampere University Hospital's Children's Clinic were enrolled in the study period between March 1 and June 30, 2005. Approximately half of them were estimated to have gastroenteritis caused by rotavirus. From 17 children in nine cases both blood and stool samples were collected and processed for testing. In eight cases only blood sample could be obtained. Blood samples were allowed to coagulate and the serum was separated from the clot. RNA was extracted separately with the QIAamp RNA mini kit (QIAGEN) and rotavirus RNA was detected with a nested RT-PCR specific for the VP7 gene, and typed by nucleic acid sequencing from the PCR products [5].
Results from the nine cases with both serum and stool samples are presented here. Six out of these cases were stool positive and three stool negative by rotavirus RT-PCR. Rotavirus RNA could be detected from five serum samples of the six stool positive cases. The types identified in the serum showed complete agreement with the types in the stools: G1 (1), G4 (3) and G9 (1). In the remaining stool positive case, even though rotavirus type G1 was detected from the stool sample, the serum was negative by RT-PCR. No rotavirus was detected in the serum of the stool negative cases.
In this study it was demonstrated that rotavirus could leak into the blood circulatory system during acute rotavirus gastroenteritis. However, the clinical importance of this finding and the role of RNAemia in the pathogenesis of rotavirus infection still remain unclear. This study may potentially act as a pilot study to a larger project examining the spread of rotavirus in other organs.

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MICROBIOLOGICAL INSPECTION OF MINERAL WATER BY REDOX-POTENTIAL MEASUREMENT

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During our work we based microbiological examination of mineral water on redox-potential measurement. In the examinations, the total number of microbes in mineral water was modeled with *Bacillus* spp. and *Acinetobacter lwoffi*. In both cases, calibration curves in TSB media were defined, and the TTD-lgN relations were linear. Using the calibration curves, the number of microbes in several commercial mineral water products has been determined, by applying the membrane filter method on 100ml of the water samples. The results were verified using regular membrane filter methods, and adequate conformity was found. To substitute membrane filtering, the examinations were also carried out by putting electrodes into the flasks of mineral water directly. In this case, straight media were placed in the flasks. The measurement was verified by using regular membrane filtering methods, and adequate conformity was found. The method was also used to demonstrate special types of microorganisms, these were: *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis*. The measurements were done using standard, selective media. The calibration curves of these microorganisms were defined (lgN-TTD), which were in all cases, linear. The typical time-redox-potential curve of the examined bacteria was determined, upon which the possibility of identifying the microflora of the water increased. The selectivity of the method was verified, and was found to be adequate. As a con-

clusion, we have found that by using redox-potential measurement, the number of microorganisms, the presence or absence and the number of undesirable bacteria in water is determinable.

PROMOTER METHYLATION AND CHROMATIN STRUCTURE IN THE REGULATION OF HUMAN INTERLEUKIN-10 GENE EXPRESSION

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Human IL-10 (hIL-10) promoter in contrast to its mouse counterpart seems to be restricted to professional cells of the immune system. However, local IL-10 production contributes to the escape mechanism of many human tumors. Since squamosus cell carcinomas of the skin and the mucosa are of keratinocyte origin, the regulation of hIL-10 in non-transformed and transformed keratinocytes was studied. Promoter methylation is an important epigenetic mechanism governing expression of mammalian genes. In this study, we analyzed the CpG methylation pattern of hIL-10 promoter with the bisulfite sequencing method. Furthermore, we examined the hIL-10 transcription with RT-PCR, and we investigated histone-3 (H3) and H4 acetylation with chromatin immunoprecipitation (ChIP). We compared cell lines of keratinocyte origin with peripheral blood mononuclear cells (PBMCs) capable of hIL-10 production.
There are six CpG dinucleotides in the proximal 0.5 kb of the hIL-10 promoter at nucleotide positions -378, -357, -355, -325, -190, -113 relative to the transcription start site. In PBMCs all six CpG sites tended to be unmethylated (0-25% methylation), while in non-transformed keratinocyte cell lines these CpG sites displayed a methylated profile (75-100% methylation). In transformed keratinocyte cell lines certain CpG sites became unmethylated, but 100% methylated CpG at position -113 and the absence of IL-10 transcription was consistently found in all keratinocyte cell lines.
The hL-10 promoter related histones proved to be deacetylated in non-transformed keratinocytes. In contrast, PBMCs displayed acetylated histones next to the hIL-10 promoter.
In conclusion, IL-10 gene is silenced probably via epigenetic mechanisms in human keratinocytes. However, hIL-10 promoter methylation may become heterogeneous during carcinogenesis.

ISOLATION AND ANALYSIS OF HIS4, A POTENTIAL SELECTION MARKER IN A *PICHIA* SP.

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Methanol-assimilating yeast species have a number of advantages when used for expressing heterologous proteins: the applicability of the general microbiological techniques for propagation, the availability of strong and easily inducible promoters and the similarity of posttranslational modifications to that of higher eukaryotes being among the major ones.
A new methylotrophic strain isolated in our institute is being developed into an expression host. In order to identify cells that received DNA after transformation, a marker conferring a phenotype that is easy to select is required. The poster reports on the isolation and the basic characterization of the *his4* gene from *Pichia sp. 159*.

Yeast histidinol dehydrogenases were compared at the amino acid level, and conserved motifs were identified. Degenerated oligonucleotides were designed accordingly and used as PCR primers to amplify segments of the *his4* gene from a genomic DNA template. One pair of primers led to productive amplification. The PCR product with the expected size was purified and inserted into a sequencing vector. The sequences of the inserts from three independent transformants were determined and used to design gene-specific primers, which were applied to amplify additional segments of the structure gene, the promoter and the 3' non-translated region by one-sided PCR. Finally, the entire gene was reconstructed from three segments amplified with Pfu polymerase. The coded protein exhibits the highest similarity to the histidinol dehydrogenase from *Pichia pastoris* (66% identity). Despite the considerable differences, it complements the *his4* mutation in *P. pastoris* GS115. The expression of the isolated gene seems to be under the general amino acid control, similarly to the *his4* in *Saccharomyces cerevisiae*.
The isolated gene was used to build plasmids for the investigation of promoter activities in a heterologous host.

HUMAN PAPILLOMAVIRUS IS MORE PREVALENT IN ORAL LEUKOPLAKIA THAN IN ORAL SQUAMOUS CELL CARCINOMA

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Mucosal human papillomavirus (HPV) types are able to infect the mucosa of the upper respiratory tract, and consequently may play a role in the development of oral precancerous and malignant lesions. Our objective was to determine the prevalence of HPV infection in oral lichen planus (OLP), oral leukoplakia (OL) and oral squamous cell carcinoma (OSCC).
Forty-six patients with OLP, 14 with oral leukoplakia and 40 with OSCC were enrolled in the study. The presence of HPV DNA was investigated in exfoliated oral mucosal cells collected from the site of OLP and OL or tumour tissue in case of OSCC, and was compared to the HPV status of the normal mucosa of the same patient. For the detection and typing of HPV, we used consensus nested MY09-MY11/GP5+-GP6+ PCR and restriction enzyme digestion with *Rsa*I and *Mse*I.
HPV was detected in 7/46 (15.2%) , 7/14 (50.0%) and 4/40 (10.0%) of samples from OLP, OL and OSSC, respectively. Exfoliated cells from the normal mucosa were HPV negative, except for one OSSC patient. HPVs found were mainly high risk types (in OLP one HPV11, four HPV16, one HPV18 and one HPV55, in OL six HPV16 and one HPV18, while in OSSC two HPV16, one HPV31 and a HPV11-HPV13 coinfection). These results show that HPV was detected almost exclusively in lesions, suggesting that they can be associated with oral premalignant and, less frequently, malignant diseases. Based on our prevalence data we hypothesized that HPV can be connected mostly with the development or maintenance of precancerous lesions, and its role in oral tumourgenesis seems to conform with the "hit and run" hypothesis.
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SPECIAL CHARACTERISTIC „EGRI LEÁNYKA" WHITE WINE PRODUCTION BY HABITAT-SPECIFIC YEASTS

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The use of starters has a long history in our country. Between 1901 and 1925, Requinyi and Soós started to collect the yeast strains of the historical wine-districts, and later used them as starters, to insure the safe process of fermentation. In spite of this, the use of Hungarian starters has been increasingly overshadowed in the last years. Several starters can be found on the market, but these products are not domestic starter yeasts. Imported yeasts can make our wines shallow and the multiplication and wild growth of these strange yeasts is undesirable in wineries.
Our aim was to create new, domestic starter-cultures from the yeasts originating from the wine district of Eger and Demjén. We also wished to create constant-quality individual Hungarian wines with a land specific taste. In our experiment we isolated yeasts from the Leányka type grapes during different periods of fermentation. We created stock-cultures from them, and made morphological, physiological and molecular-biological characterisation. Afterwards, the pre-identification and identification of the selected yeasts was made. We selected the potential starter candidates from the identified species, which were tested in microvinificated and small-scale experiments. We examined three types of yeast (SIHA, Laffort, Enoferm) on the market and a spontaneously fermented item, as control. We made routine and instrumental analytical examinations of the items during the process of fermentation as well as organoleptic examinations. Three types of yeast (G30, E38, J20 - L1 in mixture culture) proved marketable in the case of the Leányka against the foreign controls.

ESTABLISHMENT OF *AGARICUS*–PATHOGEN COLLECTION

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The mould pathogens of cultivated *Agaricus bisporus* cause considerable loss of product and profit in Hungary. There are no quantitative details of growth diminishment and data about economic damages caused by microparasites in our mushroom cultivation but the observation is based on several years' grower's experience that pathogen moulds are responsible for 80% of cultivation problems. The most important mushroom pathogens are *Verticillium fungicola*, *Mycogone perniciosa*, *Dactylium dendroides*, *Trichoderma agressivum*. Moreover, we have to take into consideration the importance of the so-called competing moulds e.g. *Scopulariopsis fimicola*, *Papulospora byssina*, *Botryotrichum piluliferum*, *Penicillium spp*., *Mucor spp*., *Aspergillus spp*., and many other species, too, because their important changes in time and space. The little knowledge about the occurrence and importance of particular pathogens strongly hinders the development of more effective protection technologies. The aim of our work was to establish a nationwide regular collection of the pathogens of cultivated *Agaricus* as a first step towards a new protection technology. We would like to deposit the representatives of the collected species in the National Collection of Agricultural and Industrial Microorganism (NCAIM) and, as a result, our work would be useful for international research.
The methodological aspects of the collection and maintenance were clarified in the preliminary step. Infected fruit-body, compost and casing soil samples were collected from five different localities in Hungary. Four media (potato-dextrose agar, glucose-peptone agar, cellulose agar, and Martin-agar) were used for isolation, carried out both directly and with dilute spore suspension spreading. After the

preliminary identification of isolates, monosporic cultures were produced, which were maintained in four different ways: refrigerated agar slant cultures, cut mycelial agar-disks in micro test tubes, cultures grown on sterile oat bran, and deep-frozen spore suspensions.
The 145 mould isolates belonged to nine species mentioned as pathogens in the literature. *Dactylium dendroides* proved to be the most frequent among them and, in spite of literary data, we can notice that *Verticillium fungicola* was starting to efface. The deep frozen spore suspensions were found to be most the appropriate method for the maintenance of isolates. Agar slant cultures and oat bran cultures were found to be more suitable for proliferation techniques.
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DIAGNOSIS OF THE „LA FRANCE ISOMETRIC VIRUS" (LIV) IN THE CASE OF *AGARICUS BISPORUS*

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The LIV („La France Isometric Virus") disease of the white button mushroom has been known for years and caused considerable fall in production in many countries (Great-Britain, the Netherlands, Canada, the United States of America, etc.). The presence of the virus has not been examined in Hungary up to now however, we have met allusive symptoms of virus infection, but were not able to detect the virus itself. The increasingly high-volume of domestic production explains the comprehensive examination of this problem. The other important reason for the research of the LIV mushroom virus is that the presence of the LIV can be checked in the mushroom spawn and LIV exempt spawn can be produced if it is necessary. Reference virus and virus free button mushroom were obtained from ATCC (ATCC 18.801, etc.). PCR primers were designed based on the sequence data of the virus. RNA was extracted from the spawn cultures, and in the course of agarose gel electrophoresis a distinction could be made between the virus infected and pure samples. An RT-PCR approach with the designed primers has been optimised to get the most reliable results. Thus a fast diagnostic technique could be achieved with these primers based on the investigation of either the mother spawn or sporophores from the symptomatic fruiting bed. Cloning and sequencing of the differentiating RT-PCR bands is underway in order to prove our results by sequence data.

INVESTIGATION OF POPLAR LEAF-RUST SPECIES IN HUNGARY

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Traditionally the main aim of poplar breeding and selection is the tolerance of or resistance to leaf-rust species. In the Hungarian poplar stands, *Melampsora* species cause rusts on leaves. One of the most important species of the genus is *Melampsora larici-populina*, but there is *Melampsora allii-populina*, which could also be harmful. The problem is most prominent in nurseries or mother stands, and it could result in early leaf-losing, restrained growing and finally an open way for other more serious damages, for example bark-cancer caused by *Dothichiza populea Sacc. & Briard*. More than 400 poplar leaf-rust samples were collected from several parts of different Hungarian poplar areas with the aim of identifying them. Microscopical identification was done following the morphological

analysis of uredospores and paraphysis. As a result of the morphological identification we can say that in Hungary the most important poplar rust species are *Melampsora larici-populina Kleb*. and *Melampsora allii-populina Kleb*. Moreover, we were interested in the plants' response, the difference between tolerance of the different poplar varieties to the disease. There is an easy laboratory method – leaf-disk method - to investigate the poplar clones' behavior to rust infection based on fenotypic response. Our result was more than finding two different susceptible groups, because as a result of morphological identification – we found only two different species -, and with the investigation of different poplar varieties' response – we found more than two different susceptibility groups – thus it was necessary to continue our work. We used uredospore morphometria to identify the species and also found more than two different groups. Nowdays there are several molecular methods to investigate the genotype of the poplar varieties, starting with isozyme analysis and now there are many good molecular techniques to investigate the genom of the plant. However, there are only some investigations to identify rust species with the same technique. Through our work we started to use a molecular analysis to characterise *Melampsora* species. DNeasy Plant Mini Kit (Qiagen) was used for DNA isolation. The quality and quantity of DNA was controlled on agarose gel and this was the template DNA through the molecular analysis. In the present report we would like to summerize the results of our investigations using different methods to decide which *Melampsora* species or races (?) are in the Hungarian poplar stands, nurseries and mother stands causing leaf rust.

IRRITATING DERMATITIS IN HUMANS CAUSED BY LARVAE OF A *TROMBICULA* SP. AT LAKE VELENCE

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Several cases of strongly itching rashes of unknown origin in humans were reported in July 2004 from Velencefürdő in Hungary. The rashes affected mainly the inguinal areas, the armpits, the belly, the neck and the scruff, the skin behind the ears and the area under the breasts in women. The number of wheals increased from 2-3 to 30-50 within a day. Similar phenomena were observed in the same period of the previous year. A sample taken from a new patient was examined at the parasitology laboratory of the Fejér County Institute of the Hungarian National Public Health and Medical Officer's Service (ÁNTSZ). The object found in the sample was a red-coloured mite larva. During the examination of the affected lakeside area, orange and red coloured mites of approximately 0.5 mm were found in the undergrowth. It turned out from the laboratory examinations that these mites belonged to the genus *Trombicula* (chigger or red bug).

MICROBIOLOGICAL FEATURES AND COMPARISON OF DIFFERENT TYPES OF TOBACCOS DURING FERMENTATION

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Microorganisms associated with tobacco leaves all come from the soil but the quantity and quality of the original microbiota can change during fermentation and storage.

Increased mechanization and instrumentalization reduced the length of the fermentation process, and the environmental conditions became more regulated and more easily controlled.
In consequence of these, it became timely again to clarify that, with altered circumstances, what microbiological and biochemical changes are the conditions given for.
The aim of our work is to study and compare the microbiological changes during preparation for fermentation, and fermentation of Burley (air-cured) and Virginia (flue-cured) type tobaccos grown in Hungary .Virginia type tobaccos differ from Burley tobaccos not only in the curing method, but they are also completely different in botanical, physical and chemical characteristics.
The samples originated from V-Tabak Hungarian Tobacco Manufacturing Company (Szolnok) and were grown in 2004. Sampling took place from bales at the threshing line from the blending bin after the redrying step and after a short fermentation period. Both samples were fermented in laboratory conditions for five months. During fermentation the environmental factors (pH, temperature, water content and humidity) were measured.
The colony forming units of mesophilic, aerobic microbes were determined at 30°C by plate-count method on trypton-glucose-yeast extract agar, incubated. The number of mesophilic, aerobic, spore-forming bacteria was obtained by similar way after the heat treatment (80°C/15 minutes). The determination of colony forming units of moulds and yeast was carried out on rose-bengale agar, and were incubated also at 30°C. The most frequently occurring moulds were isolated after fermentation. The examination of these moulds was carried out by microscopic method to obtain their genus and species. The isolated strains were identified as *Aspergillus* and *Penicillium* species. Thom and Rhaper's method was used for their identification.

ADHESION OF LACTIC ACID BACTERIA TO CACO-2H CELLS – POSSIBILITIES FOR DETECTION

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Probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host: may enhance the resistance to infections, alleviate lactose intolerance, reduce the risk of food allergy and colon cancer, etc. The ingested probiotic bacteria are able to reach the intestine and colonize it by adhering to intestinal mucosa. Many of the probiotic bacteria are lactic acid bacteria in a broad sense, particularly the species of *Lactobacillus* and *Bifidobacterium* genera. Selection of potential candidate probiotic bacteria is a multi-step process, which includes the investigation of the ability of adhesion to intestinal cells. Adherence is usually tested using human cells lines. This *in vitro* method is quick, simple and enables the screening of a large number of strains for adherence characteristics. There are several methods to detect adherent bacteria. The most frequently used techniques are: microscopic enumeration, plating and application of radiolabeling, bacteria-specific antibodies or fluorochromes.
In this study, we screened 10-10 strains from the genera *Lactobacillus* and *Bifidobacterium* in respect to their adherence to the Caco-2H cell line. Adherent bacteria were detected using three different methods: (1) Gram staining and cell count, (2) plating and (3) staining by the fluorescent dye hexidium-iodide and image analysis. We investigate the adhesion capabilities of the bacterium strains, and also evaluate the efficiency of the different detection methods.
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GENETIC DIVERSITY OF *TRICHODERMA* STRAINS ISOLATED FROM WINTER WHEAT RHIZOSPHERE IN HUNGARY

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Species belonging to the filamentous fungal genus Trichoderma are of great economic importance as sources of enzymes, antibiotics, plant growth promoters, as well as degraders of xenobiotics and as commercial biofungicides. About 40 taxa of Trichoderma have been described until now, but based on the number of described taxa in the Hypocreales order with Trichoderma anamorph morphology, the actual number is expected to grow in the next years. The investigation of mycologically unexplored geographic areas might also lead to the description of new species.
Recently, we have undertaken a study on the local biodiversity of Trichoderma in Hungary. A total number of 117 Trichoderma strains were isolated from roots of winter wheat: 19 test holes of 5 agricultural fields in southern Hungary have been sample, in January 2002. The isolates were examined both with morphological, physiological and molecular techniques. Data were collected by measuring the structure and shape of conidiophores, phialides and conidia, furthermore, colony extension measurements on potato dextrose and corn meal agar media have been carried out. Sequence analysis of the internal transcribed spacer (ITS) region was applied for the investigation of molecular diversity. No variability could be detected in the 5.8 S rRNA gene within the genus Trichoderma, but considerable variations occurred in the ITS 1 and ITS 2 regions, that are suitable for the identification and separation of the different species and for the recognition of the phylogenetic relationships among the isolates. Based on the currently suggested species concept of the genus [1], 94 isolates were identified as members of section Pachybasium B including 44 T. harzianum and 3 T. tomentosum (clade Lixii/catoptron), 32 T. virens (clade Virens), 10 T. rossicum (clade Stromatica), 4 T. brevicompactum (clade Lutea) and 1 T. spirale strain (“Lone lineages”). Eighteen isolates represent two species of section Trichoderma, clade Rufa:T. atroviride (9 strains) and T. ovalisporum (9 strains), and five isolates were identified as T. longibrachiatum (section Logibrachiatum).

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RELATIONSHIP BETWEEN TAXONOMIC POSITIONS AND BIOCONTROL PROPERTIES OF *TRICHODERMA* ISOLATES FROM HUNGARY

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Environment-friendly agriculture using less amounts of chemicals in plant cultivation is a general need in developed countries. One of the important steps in the realization of such sustainable agriculture is the application of biocontrol agents for plant protection. Certain species within the filamentous

fungal genus *Trichoderma* are potential candidates to be applied in biological control, as it was demonstrated in a great number of previous reports.
Ninety-six *Trichoderma* strains isolated from roots of winter wheat grown in agricultural fields of southern Hungary were examined as potential biocontrol agents. They belong to 9 different species: *T. harzianum* (40 strains), *T. virens* (27 strains), *T. atroviride* (10 strains), *T. longibrachiatum* (5 strains), *T. rossicum* (4 strains), *T. tomentosum* (3 strains), *T. brevicompactum* (3 strains), *T. ovalisporum* (3 strains) and *T. spirale* (1 strain).
The biocontrol properties were tested *in vitro* against *Fusarium culmorum* NRRL 29371, a significant pathogen of wheat, both on yeast extract glucose (YEG) and minimal media (MM) at 25 °C. *In vitro* confrontation assays performed on agar plates are generally applied for the evaluation of antagonistic abilities of *Trichoderma* strains, as results of these assays proved to correlate well with *in vivo* studies. A direct confrontation assay was applied for recording the inhibition effect, which was expressed as the value of biocontrol index (BCI) calculated from the ratio of the area of the *Trichoderma* colony and the total area occupied by the colonies of *Trichoderma* and the plant pathogen determined by image analysis. After collection of biocontrol data, relationship between the biocontrol efficiency and the taxonomic position was examined. Most of the strains intensively antagonized the plant pathogenic fungus and the distribution of BCIs characteristic to the members of a population showed optimum-like profiles on both media. The BCIs ranged from 13 to 60 on YEG, while they were between 28 and 52 in the case of MM. The *T. atroviride* isolates showed the best antagonistic properties on YEG with BCI values higher than 47. High BCIs ranging from 49 to 52 were obtained on MM also for certain *T. harzianum, T. virens* and *T. ovalisporum* strains.
Acknowledgement: This work was financially supported by the research grant NKFP OM-00083/2004.

PREVALENCE AND MOLECULAR BIOLOGIC CHARACTERIZATION OF ZOONOTIC PROTOZOA, *GIARDIA DUODENALIS* IN HUMANS AND ANIMALS WITH SPECIAL REFERENCE TO LABORATORY DIAGNOSTIC AND EPIDEMIOLOGIC ASPECTS.

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The flagellate protozoan *Giardia* is the etiological agent of giardiosis, one of the most prevalent and widespread intestinal diseases in humans and several vertebrate animal species worldwide. Taxonomy of the genus is mainly based on morphology and, only recently, genetic evidence. According to these criteria, six species have been recognized in the genus *Giardia* in mammals until now. *G. duodenalis* is the only species found in humans as well as in other mammals, including domestic and farm animals such as dog, cat, cattle, pig and sheep. New evidence shows strong support for homologous isolates from infected humans and houshold dogs and cats supporting their zoonotic potential. Infection and potential cyst shedding contaminates the environment for all mammals, including humans.
The prevalence of *Giardia* infection in houshold pets over wide geographic domains is less-known in Hungary. In a preliminary investigation at the 'Johan Béla' National Center for Epidemiology, National Reference Laboratory for Diseases Caused by Intestinal Protozoa of the Department of Parasitology, we studied the prevalence of *Giardia* infection in faecal samples of dogs originating from kennels. The study period was from February through July 2005. The samples were analysed micro-

scopically by direct Lugol-staining examination, and zinc sulphate flotation for the detection of *Giardia* cysts. Furthermore, the technicians were trained to evaluate faecal samples using a *Giardia lamblia* Ag-specific ELISA coproantigen test (ProSpecT Remel, USA). Every dog from which a faecal sample could be obtained during the study period was classified according to to age and breeders. Seventy faecal samples were processed using both analytical tests. A total of 52.9 % of the samples were positive by the ELISA test. Distributing the sampled population by animal age (0-2 months to >1 year), there was a significant decline in the infection rate with increasing age of the animals sampled. There was a great difference in the sensitivity of the microscopic examination (7.14 % of the samples were positive) and the ELISA test in the detection of *Giardia* infection. In order to investigate the genotypes of *G. duodenalis* originating from kennel dogs in Hungary, a nested PCR specific for the *Giardia* 18S-rDNA was introduced. The nucleotide sequences of the isolates showed a 98 % base pair sequence similarity to *G. duodenalis* dog genotype (GenBank accession number: DQ112665 of the isolate HuNCE117d; DQ118557 of isolate HuNCE 145d; DQ118558 of the isolate HuNCE147d, etc) and one of them (DQ112665) showed 96 % base pair sequence similarity to *G. duodenalis Homo sapiens*.
The preliminary investigations indicate that *Giardia* is a common parasite in dogs in Hungary. Therefore, there is a significant public health need in Hungary to determine the *Giardia* infection rate in dogs, especially in pets, living in close proximity to humans and other animals.

HIV-1 PROTEASE INHIBITION ACTIVITY OF ALKALOIDS ISOLATED FROM *HYMENOCALLIS* X *FESTALIS*, *SPREKELIA FORMOSISSIMA* AND *LEUCOJUM VERNUM*

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Alkaloids isolated from plants of the Amaryllidaceae family exert different biological effects (cell growth inhibition, antitumor- and antiviral activity etc.). We studied the antiretroviral effect of eight different alkaloids isolated from *Sprekelia formosissima*, *Hymenocallis x festalis* and *Leucojum vernum* of the Amaryllidaceae family using HIV-1 infected MT4 human lymphoma cells. Decrease in virus production was seen with lycorine, homolycorine, trisphaeridine and haemantamine.The therapeutic index (TI50) of active compounds was 1.9, 1.8, 1.5 and 1.3, respectively. Incubation of AMV (Avian myeloblastosis virus) reverse transcriptase in the presence of trisphaeridine, lycorine and haemantamine did not inhibit the activity of the enzyme. HIV-1 protease inhibitory activity was tested in a microtiter plate fluorescent assay using recombinant HIV-1 protease (expressed in *E. coli* and purified by affinity chromatography) and fluorogenic substrate. Acetyl-pepstatine served as a positive control. The time-dependent increase of fluorescence intensity was monitored in every 10 s for 30 minutes. Initial reaction speed, expressed as fluorescence units per minute, was obtained by least-squares analysis of the initial phase of the reaction. Kinetic parameters were calculated from the Michaelis-Menten equation. Lycorine showed the most pronounced protease inhibition among the alkaloids tested. Haemantamine and des-N-methyl galantamine proved to be ineffective.

UTILIZATION OF AM FUNGI IN COUNTERBALANCING THE SOIL SICKNESS OF FRUIT TREES

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Soil sickness – in other words, specific replant disease - can appear if an orchard is replanted with the same or closely related fruit species at the given place. Surface and activity of root hairs – affected by disease – decrease and consequently the growth and yield of trees get considerably worse.
Our aim was to isolate and select such mycorrhiza fungi that are able to counterbalance soil sickness similarly to thermo-therapy and chemical treatment, but without the drastic effects of these treatments. Studies were carried out by 5 different soils, 12 AM fungus strains, in pot experiments, with almond seedling as test plant.
Existence of the disease was verified by biological test of soil sickness in case of each soil. Besides the general positive tendency of mycorrhisation, 3 AM fungus strains considerably increased the growth of seedlings at a rate similar to fungicide and thermo therapy treatments.
The effectiveness of mycorrhizal treatments differed by soil types. In the case of soil originating from Érd Elvira, the effect of all the three fungus strains was significant. This effect can be considered biologically well established as well, because the competitiveness of these three AM fungus strains were confirmed in comparison with indigenous species.
In the results of root-colonization studies some spontaneous mycorrhizal infections were found in spite of chemo- and thermo therapy. The examination of presumable reasons needs further widespread study. In conclusion it can be stated on the basis of our results that there is a possibility to control replant disease in an environmentally friendly way. In order to get consistent results and introduce them into practice, the work has to be continued involving other microorganisms as well.
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PHYLOGENETIC ANALYSIS OF HEPATITIS B SEQUENCES DERIVED FROM HUNGARIAN VIRUS-CARRIERS

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Hepatitis B is classified as Hepadnavirus. The modes of the HBV transmission are sexual, parenteral and perinatal. HBV may cause chronic infection. The hepatitis B genome can be divided into eight genotypes (A-H), based on an intergroup divergence of more than 8% in the complete nucleotide sequence. These genotypes show a characteristic geographical distribution. One of the three aims of this study was to determine the dominant HBV genotypes in the Hungarian population. Another aim was to investigate an intrafamily and a nosocomial outbreak and the third goal was to analyse the nucleotide sequence of viruses derived from vaccinated HBsAg positive carriers. Altogether 65 samples were tested for HBV-DNA with nested PCR, and a segment including the pre-S1 region of HBV genome was sequenced. The nucleotide sequences were translated to amino acid sequences and a phylogenetic tree was constructed. Most of the Hungarian sera tested were similar to genotype D, although we found differences from the published sequences. In the family outbreak the sequences of the three viruses were completely identical, and could be clustered into a group similar to genotype D. In the nosocomial outbreak two sera proved to be similar to genotype A. In 29 cases out of 31, the sequences were clustered into genotype D. The sequences were very similar, the source of the infection of these 29 cases might be the same. Testing the sera of HBsAg positive children, who were vaccinated at birth, several point-mutations were identified. Some of these mutations caused amino-acid

substitutions and some were silent. None of them were identical with any of the sequences of the immune-escape variants published.

RELATIONSHIP BETWEEN THE INFECTIVITY OF ARBUSCULAR MYCORRHIZAL FUNGI AND SOIL NITROGEN NUTRITION

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Arbuscular mycorrhizal fungi (AMF), the important root symbionts should be regarded as a vital component of terrestrial ecosystems. AMF can significantly enhance the water- and nutrient uptake of host plants, plant health, and also their ability to tolerate environmental stresses (drought-, salt-, toxic element etc.). The long-established soil conservation and high available concentration of fertilizers in the soil may inhibit AM formation. When developing a new soil management form for sustainable agriculture, the role and functions of the soil microbial community should be considered. The aim of this study was to investigate the effect of various nitrogen treatments (0, 50, 100, 150 mg NH_4NO_3 kg^{-1} or 0, 150, 300, 450 kg NH_4NO_3 ha^{-1}) on the rate of *Glomus mosseae* colonization (infectivity) in pot experiments using maize (*Zea mays L.*) and bean (*Phaseolus vulgaris L.*) as test plants in a pot trial on a calcareous chernozem soil (Nagyhörcsök). Root samples of host plants were collected at the age of 10 and 20 days. The entry point number (apressoria), the frequency (F%) of mycorrhizal infection and the quantity of the arbuscules (A%) in the roots were estimated. The root colonization of AMF was universally high in both host plants and at each soil treatment. The frequency of infection, amount of arbuscules and appresoria enhanced with the increasing age of bean or of maize. The properties of root colonization showed a close correlation with the differences in N responses and with the readily available N content of the soil measured after harvesting of the 10-day-old hosts. These parameters decreased with increasing amount of N-treatment in the soil. The number of appressoria and the arbuscule content of the roots indicated the effect of nitrogen treatments more sensitively than the frequency of infection. The number of entry points showed positive correlation with the plant biomass production at each 10- and 20-days old plant. The number of AMF apressoria is an important property not only for evaluation of infectivity but also of effectiveness of AMF species. Measuring of the early AMF infection can be used as a bioindication of soil fertility status.

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APPLICATION OF AMF STRAINS FOR ENHANCEMENT OF EFFICIENCY BIOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL

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Pollution of the biosphere with toxic metals and other chemicals due to anthropogenic activities poses a major environmental and human health problem Traditional physico-chemical methods of cleanup are often difficult, expensive and inefficient. The phytoremediation, an environment-friendly technology, includes lots of methods (e.g. phytodegradation, phytoextraction, phytostabilization, rhizofiltration), using plants for removal, transfer, stabilization and degradation of contaminats in soil, sediment and water. Arbuscular mycorrhizal fungi (AMF), the important root symbionts should be regarded as a vital component of phytoremediation systems. AMF can significantly increase the water-, and nutrient uptake of plants, but can also increase their ability to tolerate stress conditions. The aim

of this work was to study how the AMF inoculums -*Glomus mossseae* and a mix inoculum which contained various *Glomus sp*. strains, tolerant to metals-affected the metal uptake of grass hosts including phytostabilization. A field trial was set up in an industrial area of a metallization factory (Hungary) with seven heavy metal stress tolerant strains isolated from metal contaminated soils. In the industrial soil, among the various metals, total Cd, Hg, Ni, Cr, Cu and Pb concentrations exceeded the permissible limit for soils several times. The experiment was set up in split plot design in four replicates with a plot-size of 4 m^2. Various grasses, resistant to drought and trampling-(*Lolium perenne*), (*Poa pratensis*), (*Festuca rupicola*), (*Festuca heterophylla*), (*Festuca rubra*), (*Festuca ovina*)- grew on the site. As an effect of inoculation, the AMF inoculums significantly decreased the Cd, Pb, Sr, Hg and Ni uptake of the shoots, as compared to the non-inoculated treatments. Zinc concentrations in shoots were not affected by inoculum of *Gl. mosseae*. Manganese uptake was enhanced by inoculation as compared to the non-inoculated grasses. Copper uptake of grass shoots was less affected by AMF inoculation. According to the results obtained on heavy metal tolerant AMF, strain collection can be used in phytoremediation of contaminated areas, as well as in diminishing the amount of heavy metals entering the food chain (soil-plant-human).

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CHARACTERIZATION OF CAROTENOID OVER-PRODUCING *XANTHOPHYLLOMYCES DENDRORHOUS* MUTANTS

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Astaxanthin accumulating microorganisms are of great biotechnological interest. This carotenoid is an important feed supplementary compound and a very powerful antioxidant demonstrating several beneficial effects on human health. Among the natural microbial source available, red-pigmented yeast *Xanthophyllomyces dendrorhous* has been described as a promising natural source of this carotenoid. Wild-type astaxanthin accumulating yeasts contain this carotenoid in a concentration of about 200 to 300 g/g dry yeast [1]. There is therefore continuous interest in elevating the pigment production to an economic level.

In the present study, cells of three different, wild-type *X. dendrorhous* isolates were subjected to successive Co60- and UV-irradiation, in order to enhance the carotenoid production. Colonies showing altered pigmentation have been isolated from non-selective plates; seven of them were selected for further experiments. Selection was based on cultural characteristics of wild-type and mutant strains: they have been compared on solid media and in liquid culture. Following several subculture cycles, pigment production and carotenoid composition were determined spectrophotometrically and by HPLC analysis. The greatest increase in astaxanthin production (6.7 times) was found in the ATCC 24229/S119 *X. dendrorhous* strain. Mutant strains with increased total carotenoid content but with insignificant change in the astaxanthin production have also been isolated.

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BACTERIAL COMMUNITY CHANGES AT TCE BIODEGRADATION

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Chlorinated solvents have been widely used as degreasing agents in the industry. Some of these compounds are toxic and carcinogenic. Conventional pump-and-treat technologies have limited effectiveness in remediating groundwater pollution. Stimulation of dechlorinating microorganisms is potentially the most promising and cost-effective technology for remediating contaminated sites.
Under anaerobic conditions, the stepwise reductive dehalogenation of perchloroethylene (PCE) to trichloroethylene (TCE), dichloroetylenes (DCE's), vinyl-chloride (VC), ethene and ethane has been observed. *Dehalococcoides ethenogenes* is the only known microorganism that can perform complete reductive dechlorination of PCE. Other isolated microbes can degrade PCE, but incompletely, to cis-DCE or VC only.
A laboratory microcosm experiment was set up to model the biodegradation of TCE. Two contaminated groundwater samples were selected, one of them with low SO_4^{2-} concentration (LS), the other with high (HS). Whey and molasses were added to the bottles as supplementary substrates. At day 0, 24, 54, 98, 155, 318 chemical and bacteriological parameters (e.g. *Dehalococcoides* test) were investigated. TRFLP based diversity assessment was carried out to observe the bacterial community changes. Based on the RNA and DNA investigations, we would like to compare the metabolically active community with the present community in the microcosms of the samples from day 318. It was found that both whey and molasses enhanced degradation. In case of bottles with high sulfate concentration amended with whey, neither ethylene nor ethane or methane was generated. In case of bottles with low sulfate concentration and whey, both ethylene and methane were detected. The result of the *Dehalococcoides* tests was positive for all samples. These results contradict those suggesting the presence and key role of *Dehalococcoides ethenogenes* only in full reductive dechlorination process. Separate groups are generated by RNA based pattern of LS samples amended with WHEY and MOLA and LS WHEY DNA samples. Also groups are created by HS MOLA DNA and HS WHEY samples. It can be concluded from these that in the LS WHEY microcosm the metabolically active and the present microbial population are almost the same, and the active members of the LS MOLA microcosm are also very similar. In case of HS WHEY and MOLA samples, the present microbial community is very similar, and only in HS WHEY are most of the present microbes metabolically active. In case of HS MOLA, the RNA based pattern is very far from the DNA based pattern. By creating a clone library and subjecting the clones to sequence-analysis, it seems that the most abundant microbes in the LS WHEY microcosm (the most successful) are *Leuconostoc mesenteroides*, *Clostridium* spp., an uncultured clone, *Trichococcus* sp. Presumably, *Leuconostoc* sp., *Clostridium* spp. and *Trichococcus* sp. have fermented the organic compounds of whey, that resulted in a lower redox potential and a higher hydrogen concentration in the microcosms. In this environment the dehalorespiration process can appear, which demonstrates the specific detection of *Dehalococcoides* sp.
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INVESTIGATIONS ON POSSIBLE GENETIC RECOMBINATIONS BETWEEN CENTRAL-EUROPEAN BLACK QUEEN CELL VIRUS GENOTYPES

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The significance of honey bees (*Apis mellifera* L.) is well known, not only through the honey-bee products, but also because of their pollinator role. In the course of a survey on the occurrence of bee-infecting viruses in Hungary, the presence of the Black Queen Cell Virus (BQCV) of the honey bee was also detected among others. The name of the virus derives from the dark areas on the apex of queen cells containing infected pupae. The consequence of the virus infection is the death of the queen larvae and pupae due to pupation disorders. BQCV belongs to a novel group of insect infecting viruses, the family *Dicistroviridae*, the genus *Cripavirus*.
The 30 nm BQCV particles contain a positive single stranded RNA genome, having two open reading frames (ORFs). ORF1 encodes the non structural proteins and ORF2 encodes the structural proteins. In a previous study, we investigated the phylogenetic relationship of genotypes after sequencing the (RT-PCR) amplification products of Central-European BQCV positive samples. Two BQCV specific oligonucleotide primer pairs were used to amplify two distinct parts of the genome. One of the primer pairs amplified a sequence on the ORF1 (coding non-structural proteins), the other a partial sequence of the ORF2 (coding the structural proteins). Phylogenetic trees were constructed from these partial nucleic acid sequences of Austrian, Hungarian and Polish BQCVs.
The comparison of the trees („helicase tree" and „structural protein tree") indicates that there are some viruses which have very different positions on the two trees. The location of one Hungarian BQCV is in the mixed group of Hungarian and Austrian viruses on the „helicase tree", but on the „structural protein tree", the same virus falls into a cluster of Polish BQCVs. Similarly, one Polish virus also has different positions on the two trees. Based on these observations, the possibility of a genetic recombination between BQCV genotypes arises. To reveal the position of the presumed recombination, a set of overlapping primer pairs was designed to cover the entire genome of BQCV. The different genomic regions were amplified by RT-PCR, and the nucleic acid sequences of the products were determined. The partial sequences were compiled and aligned determining nearly the complete genome of three possible recombinant genotypes. The similarity values of the different genome regions of the investigated viruses are presented.

PURIFICATION AND CHARACTERIZATION OF A COLD-ADAPTED PROTEASE FROM *TRICHODERMA ATROVIRIDE*

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Trichoderma species are asexual filamentous fungi with teleomorphs belonging to the Hypocreales order of the Ascomycota division. Members of this genus are well-known as cellulase producers of biotechnological importance and as antagonists of plant pathogenic fungi with biocontrol potential. Proposed mechanisms of biocontrol include the production of antibiotics, competition for the substrate, rhizosphere competence, induction of the defence responses in plants as well as mycoparasitism by the action of cell-wall degrading enzymes. The involvement of extracellular chitinolytic and b-1,3-glucanolytic enzyme systems of *Trichoderma* in mycoparasitism was investigated in detail,, while the extracellular proteolytic enzyme system remained relatively unknown in the case of this genus. Fortunately, in the recent years more and more attention has been paid to the investigation of *Trichoderma* proteases and their potential role in biocontrol and other processes [1]. A cold-adapted protease was isolated from the ferment broth of a cold-tolerant *Trichoderma atroviride* strain with excellent biocontrol potential. The strain was grown in the presence of casein in shaken flasks at

10°C for seven days and the culture was filtrated. The filtrate was concentrated by lyophilization and applied to a Sephadex G-100 chromatography column.
The fractions showing the highest activities at 5°C on the trypsin substrate *N*-benzoyl-Phe-Val-Arg-*p*-nitroanilide were combined, concentrated on a membrane-filter and chromatofocused on a Polybuffer exchanger 96 column with Polybuffer 74. The fractions showing the highest activities were concentrated and the purified enzyme was characterized. The molecular weight of the enzyme was 57 kDa as determined by SDS-PAGE. The isoelectric point was determined as 7.3 on Novex precast IF gels. The enzyme had a pH optimum of 7.4, a temperature optimum of 15°C and showed 66% activity of the optimum even at 5°C. Based on the inhibitory effects of distinct inhibitors, the enzyme proved to be a trypsin-like thiol protease.
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PHENOTYPIC PROPERTIES OF *KLEBSIELLA* SPECIES ISOLATED FROM BLOOD CULTURE

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Klebsiella species are frequently associated with human nosocomial infections such as respiratory and urinary tract infections and septicaemia. They are considered as important opportunistic pathogens since they primarily attack immunocompromised patients suffering from underlying diseases.
A total of 44 *Klebsiella* isolates from blood cultures of septic patients were examined for the presence of particular virulence factors such as various types of fimbriae, siderophore production and serum resistance. In (61.4%); {27/44} of the *Klebsiella*-positive blood cultures this species was the only bacterium isolated (monomicrobial infection), and in (38.6%); {17/44} it was found with other bacteria (polymicrobial infection). *Klebsiella pneumoniae* was the dominant species (88.7%); {39/44} while *K. oxytoca* was found in only (11.3 %) ; {5/44} of the isolates. Type 1 fimbria (mannose-sensitive haemagglutinin) was expressed by (50.0%); {22/44} of the isolates. Type 3 fimbria {mannose-resistant and *Klebsiella*-like *haemagglutinin* (MR/K)} could be shown on (47.7%); {21/44} of the strains. Siderophore was produced by (88.6%); {39/44} of the isolates. Enterobactin positivity was found in (86.3%); {38/44} strains, and only a single strain produced aerobactin (2.2%); {1/44}.
The level of serum resistance could be distributed into seven categories based on the counts of survivor bacterial cells during the three-hour incubation period of the experiment. An unexpectedly high proportion of the isolates (88.6%); {39/44} belonged to one of the four sensitive categories ranging from high sensitive to low sensitive. Among the five isolates (11.3%); {5/44} belonging to one of the three resistance categories, only two strains (4.5%); {2/44} could be classified into the highly resistant group. Further characterisation by capsular typing, resistotyping and molecular analysis of the isolates is planned.

CULTURABLE BACTERIAL PARTNERS OF TWO CRUSTACEAN SPECIES: *DAPHNIA CUCULLATA* AND *EUDIAPTOMUS GRACILIS*

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Crustacean species play a key role in the purification and filtration of natural waters. Bacterial communities of two different nutrition type planktonic species from Lake Balaton were studied on the basis of cultivation-dependent methods. Together with the *Daphnia cucullata* and *Eudiaptomus gracilis,* the water of the lake at Tihany was also examined.
30 individuals of *D. cucullata* and *E. gracilis* were homogenised respectively and used as composite samples. The water and the Crustacean samples were diluted and spread onto the following media: KingB, nutrient, chitin, *Cytophaga* agar media, Kidney disease medium, special oligotrophic medium and pepton-yeast extract agar and then incubated at 28°C under aerobic conditions. Isolation of bacteria happened at random, then representative strains were selected for detailed search: 54 strains from *Daphnia*, 49 strains from *Eudiaptomus* and 27 from the surface water sample of Tihany.
Fatty acids of all isolated strains were prepared and analysed by GC (HP 5890) using HP1 capillary column. After their fatty acid profile, the strains were grouped using Syntax 2000 software package and then for 16S rDNA sequence analysis representative strains were selected again: all ungrouped strains and one from each phenon. Sequence analysis was performed on an Applied Biosystems Model 310 Genetic Analyser. To evaluate the degradation capacity of the strains, different kinds of biopolymer utilisation were tested (chitin, starch, gelatine, cellulose etc). As a pathogenicity factor, hemolysine production of the strains was also studied.
Microbacterium oxydans (Actinobacteria), *Aeromonas culicicola* (g -Proteobacteria) and *Micrococcus luteus* (Actinobacteria) were common in the water and also in both Crustacean species. *Exiguobacterium aurantiacum* (Bacillaceae) and *Chryseobacterium joostei* (Flavobacteriaceae) were characteristic only in Crustacean. *Aeromicrobium* (Nocardioidaceae), *Deinococcus, Hydrogenophaga* (b -Proteobacteria) and *Serratia* (g -Proteobacteria) species occurred in the *Daphnia* sample, *Pseudomonas* (g -Proteobacteria), *Sphingomonas* (a -Proteobacteria), *Cellulomonnas* (Actinobacteria) and *Staphylococcus* occurred in the *Eudiaptomus* sample. *Stenotrophomonas* (g -Proteobacteria), *Brevundimonas* (a -Proteobacteria), *Rhodococcus* (Actinobacteria) species could be cultivated only from the water sample. Strains showed different affinity in degradation processes, starch and gelatine were the most easily degradable compounds. 86% of the strains had haemolysine.

THE EFFECT OF PENICILLIN TREATMENT ON THE EXPRESSION OF *CHLAMYDIA PNEUMONIAE* GENES

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The persistent form of *Chlamydia pneumoniae* infection may have a significant role in the development or progression of certain chronic diseases, including atherosclerotic cardiovascular and cerebrovascular damages. Inadequate antibiotic treatment of patients suffering from acute *C. pneumoniae* infection may contribute to the development of a persistent state of chlamydial infection. We examined the replication and gene expression of *C. pneumoniae* in susceptible HEp2 cells in the presence and absence of Penicillin. HEp2 cells in 24 wells plates were infected with a partially purified *C. pneumoniae* preparation, strain TW183, at a multiplicity of infection of 3. Half of the cultures were treated with Penicillin (IU/ml), the other half of the cultures were left untreated. The samples of the treated and untreated cultures were obtained at different times, 0h, 24h, 48h, 72 and 96h after infection, and proceeded to infectious bacterial titration and gene expression measurements. For the ex-

amination of the expression pattern of chlamydial genes, the following genes were included in the study: 16S RNA (bacterial house-keeping gene), GroEl (cHSP60 gene, expressed early after infection), ompA (major outer membrane protein gene, expressed in the middle of replication), omcB (cysteine rich protein gene, expressed late in infection), and the ftsK and ftsW (cell division-related genes). After RNA isolation, DNase treatment and reverse transcription, the gene expression was detected by real time PCR on Roche LightCycler 2.0 using SYBR-Green.
The bacterial titer decreased to the level of undetectability by the 48th-72nd h p.i. in the Penicillin treated cultures, but increased to $3x10^5$ IFU/ml in the untreated cultures. Following the generation of a standard curve for each gene, the mRNA expression of the chlamydial genes was quantitatively determined. The examined genes were expressed in the Penicillin-treated cultures, however the expression pattern of the genes was different from that of the untreated cultures, indicating a persistent state of the bacteria during the Penicillin treatment. These results emphasize the importance of an effective and specific antichlamydial treatment during an acute *C. pneumoniae* infection.
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IDENTIFICATION AND CHARACTERIZATION OF AN AUTONOMOUSLY REPLICATING ELEMENT FROM A *PICHIA* SP.

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Pichia sp. 159, a methylotrophic yeast strain, could potentially be developed into a new expression host; information about the required elements of a functioning expression system is therefore demanded. We report on the isolation and characterization of an autonomous replicating sequence (ARS) isolated from this strain. Parallel banks were created from partially digested *Pichia sp. 159* genomic DNA, and a pool of about 105 independent plasmid clones was subjected to repeated selection for autonomous maintenance in *Pichia pastoris*.
The selection procedure resulted in 11 independent ARS candidates. The sequences of the inserts (in the range of 320-1500 bp) were determined. Multiple comparisons of the sequences revealed several possible common motifs. ARS-125 was selected for detailed analysis because most of the potential motifs were present in this insert and because of its relatively small size. Progressive deletions from each side were created, eliminating the potential ARS motifs step by step. The ARS function conferred by the modified inserts was determined by measuring the apparent growth rate of the transformants under selective conditions, and by calculating the rate of plasmid loss under non-selective conditions. As a result, the ARS function was localized to a 68-bp region, which was further divided into a 44 and a 24-nucleotide segment. The former is extremely A/T-rich (91%) and contains 7 units of alternating AT bases. This itself, and also with its flanks, provides several possibilities for the formation of secondary structures. This segment alone exhibited full ARS functionality. Possible positional effects were eliminated by inserting this region into a different location on the plasmid, in both orientations. The 24-bp segment conferred an intermediate phenotype. The apparent doubling time of 4.2 ± 0.5 h was longer than that of a functional ARS (3.8 ± 0.3 h), but much shorter than that of the vector control (12.2 ± 1.1 h). The plasmid was lost about twice as quickly as the well-replicating ones, but at a significantly lower rate than that for the vector itself. This region carries the 8-nucleotide motif of CACT(t/g)GTT present in all 11 ARS isolates, without or with only one mismatch (5/6 isolates, respectively). It is overlapped by a somewhat less well-defined motif GG(t/a)TT(c/a)TG, which is present in 9 isolates with a maximum of 1 mismatch.
The *Pichia sp. 159* ARS does not support replication in *Saccharomyces cerevisiae*.

PCR DIAGNOSIS OF COLUMBID CIRCOVIRUS INFECTION IN HUNGARY

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Columbid circovirus (CoCV) is a member of the genus *Circovirus*. Circoviral infection in pigeons was first recorded in the USA [1] but nowadays it has been reported worldwide. In Hungary, the occurrence of CoCV was first reported in 2002 [2]. The disease affects young pigeons up to 6 months of age, but the virus can be detected also in older pigeons without clinical signs [3]. Gross pathologic changes include swelling and oedema in the bursa of Fabricious and the presence of particles resembling circovirus in the bursa have been reported [4].
Tissue samples including bursa of Fabricious, spleen, liver, blood and feather pulp were collected from 41 pigeons from 13 lofts. From further 4 lofts, individual feather samples were examined from 12 young pigeons. These samples were collected from pigeon offsprings each month starting at the age of 6–8 weeks up to 6 months. CoCV specific PCR primers were designed within the conservative region of the replicase protein (rep) gene. All of the samples were amplified by PCR. One of the samples was directly sequenced. The sequence identity between the Hungarian sample and the published CoCV sequences was 93–98%.
Our previous studies confirmed the massive CoCV infection of the Hungarian pigeon population. The majority (80%) of the Hungarian pigeon lofts, examined in this survey, was found to be infected with CoCV. Therefore, it was important to introduce a more reliable method for the exact confirmation of histopathologic diagnostic findings, and for application in live birds. Initially, we have tested the feasibility of blood samples, but this seemed to be too difficult, especially because breeders consider blood sampling inappropriate. As an alternative, we tested if feather samples from live pigeons were suitable for CoCV PCR.
In a pilot study, we examined individually different organ samples (liver, spleen, bursa of Fabricious, and feather pulp) from pigeons, in which the presence of CoCV was suspected on the basis of histopathology. The results did not show significant differences between the amount of CoCV detectable from different organs and the feather pulp. To study the dynamics of infections, individual feather samples of 3–3 registered pigeon offsprings from each of the four lofts were repeatedly examined. None of the pigeons were found infected at the first sampling, however, one month later several individuals were found positive. During this study, the CoCV positive pigeons died or were lost within 1–2 months. We suppose that CoCV-infected birds that did not return from training flights died also as a consequence of poor conditions. Thus mortality might be considered as 100%. We plan to continue this survey preferably by extending it to a larger geographical area of Hungary. (NCBI accession number: DQ019447)

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GENETIC CHARACTERIZATION OF *BOTRYTIS CINEREA* ISOLATES OF EGER AND TOKAJ WINE REGIONS

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The development of grey mould on grape berries is among the most striking viticultural phenomena with complex preconditions and implications. The filamentous ascomicetous fungus *Botryotinia fuckeliana* (anamorf: *Botrytis cinerea*) is the causal agent of the destructive grey mould (*pourriture grise*) as well as the noble rot (*pourriture noble*), an infection that results in highly prized, sweet, smooth, full-bodied, special quality wines with a most pleasant bouquet. In Hungary, the Tokaj Wine region has a reputation for the production of these great sweet *pourriture noble* wines, called „aszú". On the other hand, in the nearby Eger Wine region, it is the *pourriture grise* that usually causes serious losses. *B. cinerea* grows rapidly and gives rise to *pourriture grise* whenever the berry exudes a drop of juice from wounds such as those caused by hail, insects, birds, other fungi, or during the partial detachment of the berry from the pedicel or during the shedding of the floral parts from the ovary. On the other hand, if *B. cinerea* infects a healthy berry with a skin that remains intact even after maturation, *pourriture noble* may occur: conidia will germinate on the surface of the berry, germ tubes and fine infective hyphae will subsequently develop. Under certain environmental conditions, the fungus drains water but no other substrates from the berries, significantly increasing the concentration of all soluble compounds including sugars.
Knowledge on the size and structure of *B. cinerea* populations is essential for the effective and economical protection against grey mould as neither resistance genes of the host plant, nor fungicides can maintain their effectiveness for an elongated period of time. Moreover, it would be highly advantageous both from an academic and an economic point of view to know if there is a difference between *B. cinerea* populations causing grey mould and noble rot. Application of tools provided by recent advances in molecular population genetics and biology are crucial in gathering this information.
Based on these considerations, a first-of-its-kind study to characterize *B. cinerea* populations of two Hungarian wine producing regions was carried out. In the initial stage, 80 isolates of grapevine berry-growing *B. cinerea* from various locations of the Eger and Tokaj wine regions, respectively, were collected. Individual strains were obtained by single-spore isolation. Characterization of their genotype was done by analyzing MSB1 minisatellite, a fragment of the translation elongation factor 1 (tef1) gene sequences and by determination of the presence or absence of transposon elements.
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INVESTIGATIONS ON THE SEDIMENT BACTERIAL COMMUNITIES IN LAKE HÉVÍZ USING CULTIVATION-BASED AND CULTIVATION INDEPENDENT MOLECULAR METHODS

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Lake Hévíz is the largest natural thermal lake in Europe. Curative sludge evolved by the effect of the curative water and peat layers. The hydrogeological circumstances of the lake have changed in the 1980's, which was due to the near exploitation of karst water needed for mining. However, the lowered water output has been stabilized recently. The microorganisms, by their metabolism can be responsible for the curative effect of the water. Therefore, the knowledge of the bacterial communities of the sediment is essential. As earlier investigations showed, many of the dominantly occurring *Streptomyces* and *Micromonospora* species are able to synthesize bioactive compounds, which can be

used in the medical industry. These former studies resulted in the phenotypical description of a new cellulose hydrolyzing bacterial species, the *Micromonospora heviziensis*.
The aim of our work was to gain information about the bacterial species composition of the communities of the lake's sludge. Considering the selectivity of the microbiological methods, cultivation-based and cultivation-independent molecular techniques were used to explore species diversity.
More than 400 aerobic bacterial strains were isolated from six selective media with specific compositions. Strains were grouped primarily by the properties of their colony morphology and 99 pure cultures showing different morphology were subjected to ARDRA. The cleavage by restriction enzymes yielded 34 groups with different molecular patterns. Partial 16S rDNA sequence analysis of the group representatives was carried out. Species of high G+C (*Arthrobacter, Micrococcus, Micromonospora, Rathayibacter, Rothia, Subtercola*) and low G+C Gram positive Firmicutes (*Bacillus, Brevibacillus, Staphylococcus),* α-Proteobacteria (*Sphingobium, Sphingomonas, Xanthobacter)* and γ-Proteobacteria (*Chrysobacterium*, *Pseudomonas*) were identified. The representative strain of the most numerous ARDRA-group showed the highest (99%) sequence similarity to an antibiotic producing *Bacillus* species. Therefore, we started to investigate the phenotypical characteristics of this group. DNA was also gained directly from sludge samples. The mixed PCR product was used to create a 16S rDNA clone library. Screening of the 125 bacterial clones resulted in 39 ARDRA-groups. Sequence analysis of the representatives revealed a great diversity: 65% of the clones were identified as Proteobacteria, other representative clones showed the presence of Cyanobacteria (15%), Thermomicrobia (6%), Firmicutes (1%), Spirohetes (2%), Bacteroids (4%) and Deferribacteres (1%).
Acknowledgement: This work was supported by OTKA 43617.

DETERMINING DRY MATTER CONTENT OF PHASE II *AGARICUS BISPORUS* COMPOST BY NIR-TECHNIQUE, AS AN EXAMPLE OF CHARACTERIZING THE QUALITY OF MUSHROOM COMPOST

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Mushroom (*Agaricus bisporus*) mass-cultivation is an important high-technology agricultural practice. One of the major problems is to ensure the permanent quality of the phase II mushroom compost, which deeply influences mushroom yield. Quality cannot be ensured only by a standardized composting process, because the parameters of the raw materials always change, so the compost has to be monitored continuously. Basic parameters are dry matter-, carbon-, nitrogen- and ash content, pH, fibre fractions and the composition of thermophilic microbial population. The conventional methods for the determination of these parameters are time- and labour-consuming. The use of near infrared reflectance spectroscopy (NIR) can be an alternative method. It is quick and cheap, and gives the opportunity for on-line feedback into the composting process. Naturally this method has to be calibrated with the conventional techniques.
We began the optimization of this method by determining the dry matter content of the compost. This parameter was chosen because it can be measured easily in a conventional way, and the water-content has a high correlation with the NIR spectrum. In the first step the method was standardized for sample processing and NIR spectrum measurement. Then a calibration was made for determining the dry matter content, and the calibration was optimized. Our results showed that the regression coefficient of the calibration (R2) was above 0.95, which meant that the specified and the estimated values are highly correlated. In other words it indicated that there is a strong correlation between the dry matter content and the infrared spectra. On the other hand, we could not yet successfully predict the dry matter content of new samples.

FACTORS INFLUENCING THE WATER QUALITY OF LAKE BALATON

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Water quality of Lake Balaton generally complies with the most rigorous regulations. However, occasional unexplained contaminations represent risk to public health. Our aim was to investigate three potential sources of contamination: effluent from wastewater treatment plants (with emphasis on the efficiency of tertiary treatment), streams running into the lake and bird faeces. The focus of the research was the presence of human pathogens, including bacteria (*E. coli, Campylobacter, Salmonella*), viruses (human calicivirus, adenovirus, rotavirus, astrovirus and hepatitis A virus) and protozoa (*Cryptosporidium, Giardia*), that have been previously connected to water-related outbreaks or sporadic cases of waterborne diseases. Pathogens were detected and identified using culture based and molecular techniques.

Four different methods of tertiary treatment were compared in sewage treatment plants, where the effluent was released directly or indirectly into the lake. All effluents complied with the strict local limit values for phosphate (0.7 mg P L-1). $Ca(OH)_2$ as a tertiary treatment was the most efficient in the removal of phosphorous, bacterial and viral contaminants. Protozoa were, however, remarkably resistant to all treatments.

Water quality of surface water inlets and adjacent public beaches was analyzed and compared. The streams were highly contaminated, especially in areas where the sewage system is underdeveloped. Indicator bacterial counts repeatedly exceeded limit values for bathing water, human viruses and *Giardia* or *Cryptosporidium* cysts were present in most analyzed samples. Microbiological quality of the beaches was good to excellent, when characterization was based on routine bacterial counts. *Campylobacter* was not detected in surface water. *Salmonella* was present in one influent and the corresponding beach. Viral pathogens or protozoa, where present in the effluent were detected in the lake water as well, proved to be more persistent in this environment than bacterial pathogens. Sudden loads of microbial contamination washed in by the increased flow during thunderstorms did cause a local decline in water quality.

Bird faeces samples contained high counts of *E. coli* and faecal *Enterococcus*. *Salmonella* was detected in 6, *Campylobacter* in 9 of the 22 analyzed samples. Quality of water samples collected in areas frequently visited by birds (seagulls or swans) was excellent, though they were more contaminated than the off-shore control sample, which met even the drinking water standards. In conclusion, both wastewater effluents and surface water flows contain high counts of microbial contaminants. Viruses and protozoa were more resistant during sewage treatment or natural filtration of the water through reed, therefore these probably represent a more relevant public health risk than bacterial pathogens. There was no evidence of contamination related to bird faeces.

EFFECTS OF HUMAN PAPILLOMAVIRUS ONCOPROTEINS ON THE APOPTOSIS OF HUMAN DIPLOID CELLS

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Apoptosis (programmed cell death) has important roles in embryogenesis and defence mechanisms. Alterations of apoptotic response may lead to autoimmune diseases or tumour development. Certain oncoproteins of human oncogenic viruses were shown to modulate the apoptotic response of host cells. We studied the effects of the E6 and E7 oncoproteins of human papillomavirus type 16 (HPV-16) on the apoptotic processes of human embryonic fibroblast (HEF) cells.
For stable and uniform expression of the HPV oncoproteins, HEF cells were infected with recombinant retroviruses carrying either control vector (LXSN), or HPV 16 E6 and/or E7 oncogenes. After drug selection, apoptosis was induced either by cell confluence, or by the cytotoxic agent cisplatin. Then we tested either cell viability by MTT test or apoptotic cell death by measuring intracellular DNA content in FACS (fluorescence-activated cell sorter) analysis.
We found that HPV 16 E7 strongly induced apoptosis in confluence arrested HEF cells. Interestingly, co-expression of E6 totally abrogated this pro-apoptotic effect of E7 on HEF cells, which may be related to the ability of E6 to induce the degradation of p53. On the other hand, we showed that the presence of E6 and/or E7 in HEF cells highly increased the level of cisplatin-induced apoptosis. Our results indicate that the effects of the same oncoprotein (E6) on the apoptotic response of the same host cell may be completely different depending on the apoptosis inducing stimuli.

SUBACUTE SCLEROSING PANENCEPHALITIS IN CROATIA (1994-2004)

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Subacute sclerosing panencephalitis (SSPE) is a rare progressive neurodegenerative disorder of the central nervous system caused by persistent defective measles virus (MV). The disease occurs 5-10 years after the initial attack of measles. Onset is usually characterized by mental deterioration and myoclonus leading to a vegetative, decorticated state, coma and death within 1-3 years. Demonstration of intrathecal MV-specific antibody synthesis or detection of MV-RNA from brain tissue confirms diagnosis. We describe the five SSPE cases diagnosed in the Croatian National Institute of Public Health (CNIPH) from 1994-2004. During the 10-year period (1994-2004), five patients suspected of SSPE were reported to the Reference Epidemiology Centre of the Ministry of Health at CNIPH. Serologic testing of serum and cerebrospinal fluid (CSF) for MV and varicella-zoster virus (VZV)-specific antibodies was performed using the complement fixation (CF) test (micromethod) and enzyme-immunosorbent assay. Brain tissue samples of two patients obtained at autopsy were examined by light microscopy, electron microscopy (EM) and indirect immunofluorescence. The age at the onset of disease was 5-11 years. All patients were vaccinated regularly against measles according to the immunization schedule. Two of them had a history of morbilliform rash (unrecognized measles) at the age of six and seven months, respectively. One patient had measles at the age of 18 months. In two patients the disease started immediately after varicella infection. CSF analysis revealed IgG synthesis within the CNS with oligoclonal bands of immunoglobulins. Antibodies to MV were detected in the CSF and serum of all patients. The CF-antibody titers ranged from 1:1024 to 1:65536 in serum and from 1:16 to 1:128 in CSF. In CSF, no antibodies to VZV were found. Brain tissue samples were obtained at autopsy from two patients. In both cases, MV antigen was detected in brain imprints using IFA. In one patient, electron microscopy demonstrated intranuclear viral inclusions (MV nucleocapsids). Using RT-PCR, viral RNA was found in both patients. Nucleotide sequence analysis

showed that the viruses detected in the brain tissue belong to the wild-type MV D6 genotype 1. In four patients, the disease had a progressive course and all of them died in the period of 4-15 months after the onset. One patient had a short period of disease progression, but remained stable long thereafter. Still alive, she suffers from severe neurological defects (spastic tetraparesis, blindness and aphasia) [1].

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WHICH MICROBIOLOGICAL PARAMETERS CAN BE DIFFERENT IN THE RHIZOSPHERE OF THE GENE-MODIFIED BT CORN?

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Rhizosphere of higher plants is a unique environment, where microbiological status is highly dependent on the initiative activity of the host plants. Rhizosphere soil- and rhizobiological investigations may indicate the effect of gene-modification of the host (*Zea mays* L) through the eco-physiological changes of the root. The investigated genetically modified model corn expressed the *cry1ab* gene from *Bacillus thuringiensis* ssp. *kurstaki*. During three consecutive years, soil- and root samples of transgenic *Bt* (DK-440-BTY) and isogenic non-*Bt* (DK-440) control corn were collected seasonally. Beside the endorhiza colonisation by the arbuscular mycorrhizal fungi (AMF), the rhizosphere parameters, such as the countable microbial components (r, k, l strategist heterotrophic, oligotrophic and spore-forming bacteria, respectively) were assayed and the catabolic enzymatic activity of the total microbial biomass was measured by fluorescein diacetate (FDA) hydrolysis [1,2]. Abundance and species composition of the *Trichoderma* fungi was assessed from the outer rhizosphere.
The exudates of transgenic *Bt* corn had no apparent detrimental effect on the rhizosphere microbiota, as reported by the countable microbiological parameters. However, there was a seasonal variability recorded, depending on the studied microbial groups with different surviving strategies in the rhizosphere. Total microbial activity measured by FDA hydrolysis for the *Bt* corn was significantly higher ($P<0,05$) in comparison with that of the non-*Bt* corn in the first two years, while the same difference was not significant in the third year. Rates of endosymbiont mycorrhizal colonisation, however, were significantly lower ($P<0,05$) in the case of the Bt corn, although these differences between the total annual mycorrhizal activities of the *Bt* and non-*Bt* corn became non-significant in the third vegetation period. No difference could be found in the abundance and diversity of *Trichoderma* fungi in the soils with less rhizosphere influence. The role of altered plant-physiological properties in the different rhizobiological parameters is highlighted in the study.

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[2] Schnürer J and Rosswall T: Appl Env Microbiol **43**, 1256-1261 (1982).

INVESTIGATION OF MICE PATHOGENICITY OF *PASTEURELLA MULTOCIDA* STRAINS IN ORDER TO ESTABLISH A HIGHLY PATHOGENIC CHALLENGE STRAIN IN RABBITS

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P. multocida strains (n=12) have been isolated from rabbit autopsy from pathognomical lesions (n=4), and from the nasal mucosa of clinically healthy (n=5) or diseased (n=3) animals. Mice were challenged intraperitoneally with 5 series of tenfold dilutions and the 50% lethal dose was estimated. One strain was highly pathogenic with LD50 = 2,68 CFU, and three more were also pathogenic, LD50 being 1,42-9,4 × 10^5 CFU. Five strains showing LD50 = 1,47-4,93 × 10^6 CFU were considered as moderately pathogenic, and four with LD50 above 1,73 × 10^8 as not pathogenic at all. A set of parameters, including health status and clinical or pathognomical lesions on sampled rabbit, colony and capsular types of the bacteria, occurrence of neurological signs or systemic infection in mice, were evaluated statistically to find out if any of them could have an association with the virulence.
The circumstances of isolation were found to be important, as all isolates taken from dead rabbits clustered together, and three of them were pathogenic on mice as well. The remaining one was classified as being non pathogenic to mice, although a relatively high number of mice were killed with the concentrated inoculum containing 10^7 CFU (but none with the diluted ones). Differences in capsular serotype also showed some interaction with the circumstances. The only two capsular type F stains have been collected from healthy rabbits and were separated below 60% similarity into one cluster. These were also non pathogenic to mice, and did not produce neurological signs. The third cluster included six strains taken from ill or apparently healthy rabbits, and all were capsular type A. The lethality of these isolates to mice was heterogeneous, one non pathogenic, 3 moderately pathogenic, and two pathogenic were found. Considering these results, it can be concluded that either ill or clinically healthy rabbits could spread pathogenic or moderately pathogenic strains, and the proportion of the non pathogenic strains is not different in the apparently healthy rabbits.

STREPTOMYCETES AS PRODUCERS OF LIPOLYTIC ENZYMES

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Microbial lipases are widely diversified in their enzymatic properties and substrate specificities. According to previously published results, it seemed that members of this genus are not typical lipase producers compared to other bacteria. There are five cloned and sequenced lipase genes from streptomycetes described so far: lipases from *S. exfoliatus*, *S. albus* and *S. coelicolor* exhibiting more than 80% sequence identity in spite of their taxonomic divergence, and lipases that show no similarity to those, from *S. cinnamomeus* and *S. rimosus*. Based on the bacterial lipolytic enzyme classification, *S. cinnamomeus* lipase belongs to family I while *S. exfoliatus*, *S. albus* and *S. coelicolor* A3(2) lipases are members of family III. Our results show that the novel lipase from *S. rimosus* belongs to GDS(L) or family II of the lipolytic enzymes as well as the *S. coelicolor* putative hydrolases that were found by data base search, thus representing a third lipase family previously unrecognized in *Streptomyces*. Out of eight examined streptomycetes, the presence of this rare type of bacterial lipase gene was detected in two strains belonging to the *S. rimosus* taxonomic cluster, and in one unrelated species. To our knowledge, there is only one bacterial lipase characterized from *Photorabdus luminescens* that belongs to the same family of lipolytic enzymes. *S. rimosus* lipase gene has been located on the *AseI* B fragment approximately 2 Mb far from the left end of the *S. rimosus* linear chromosome, similar to the position of the putative *S. coelicolor* lipase gene indicating non-essential functions of these genes. Biochemical properties of this rare type of bacterial lipase might have a significant biotechnological potential due to its relatively high working temperature, pronounced stability as well as an unchanged

activity over a broad pH range. Comparison of the peptide mass fingerprints from the reduced and non-reduced overexpressed enzyme unequivocally revealed three intramolecular disufide bonds. A chemical modification approach combined with MALDI mass spectrometry identified the serine, at position 10 in the Gly-Asp-Ser-(Leu)-like consensus motif, as the nucleophilic amino acid residue. Thus, this experimental evidence supports classification of these novel bacterial enzymes.

UNFORESEEN BEHAVIOUR OF THE RABIES VIRUS IN MOUSE EXPERIMENTAL SYSTEM

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The results of rabies virus experiments are presented, which represent a part of a larger experimental project. The rabies virus has been detected using two independent techniques from the organs of mice following intramuscular inoculation. The experiments allowed the detection of the timetable of the peripheral progression of the infection in a white laboratory mice line. These results will provide basis for the continuation of the research since there are many open questions in connection with the pathomechanism of rabies. How the virus replication proceeds in the muscle cells, why no antigen presentation occurs during the time of muscular replication (peripheral immunosuppression? Non cytocidal virus replication in muscle cells?) are some of the questions. The experiments have been initiated on the basis of the recent transplantation accidents. The transplant recipients obtained rabies from organs of the donors. In addition to the corneal transplant recipients other organs, such as lungs and kidneys have transmitted the infections. The neurotropism of rabies virus is outstanding among the neurotropic viruses. This property is determining the pathomechanism of the disease and the character of the immunologic processes connected to it. The intraaxonal spread of the virus is unique and the rabies virus cannot cause viremia in the patients. It can enter the sensoric or motoric dendrites or axons at the site of the injury (probably most frequently at the neuro-muscular junctions through the acetylcholine receptors). Then the virus will be transported by yet unknown molecular mechanisms centripetally to the central nervous system. After the unlimited replication in the central nervous system, it is transported centrifugally via the trigeminal nerve. One of the transplant recipients, however, survived after a corneal transplantation, indicating that the donor has been suffering from an early stage of the rabies infection. The recipient of the kidney of the same donor died, which indicate other possible ways of the transport of the rabies virus in the human body.
The mouse experiments try to detect whether the virus might be able to be released during the intraaxonal transport into the surrounding tissues in spite of the fact that no immune response is initiated during the latency of the disease.

HOMOLOGOUS RECOMBINATION AND DNA REPAIR IN RECBC SBCBC MUTANTS OF *ESCHERICHIA COLI*: EFFECTS OF A RECG MUTATION

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RecG protein is a DNA helicase that promotes DNA strand exchange and heteroduplex extension during homologous recombination in *Escherichia coli*. Cells mutated in the *recG* gene show moder-

ate recombination deficiency and increased UV-sensitivity compared to the wild type cells. We have studied the effects of a *recG* mutation on recombinational DNA repair after exposure to UV-irradiation, on conjugal recombination, and on cell morphology in two types of *recBC sbcBC* mutants of *E. coli*. In one mutant, the *sbcB* gene was inactivated by a point mutation (*sbcB15*), while it was completely deleted (D*sbcB*) in the other. We found that introduction of the *recG* mutation in *recBC sbcB15 sbcC* background strongly reduced the efficiency of recombination and recombinational DNA repair, and caused severe defects in chromosome segregation and cell division. On the contrary, in *recBC* D*sbcB sbcC* background, the *recG* mutation had relatively mild effect on both recombination processes and cell morphology. We inferred that the *sbcB15* mutation modifies the recombinational process in a way that the process becomes more dependent on the RecG protein. It is possible that the mutant SbcB15 protein binds to recombinogenic 3' DNA ends and interferes with the processing of recombination intermediates in the absence of the RecG protein.

DEINOCOCCUS RADIODURANS: THE MOST EFFICIENT DNA REPAIR INVOLVES COUPLED REPLICATION AND RECOMBINATION PROCESSES

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Bacterium *Deinococcus radiodurans* is one of the most radiation resistant organisms known. It can sustain extreme levels of ionizing radiation that induce several hundred double-stranded DNA breaks in its genome. The key to its radioresistance seems to be a highly efficient DNA repair, however, the molecular mechanism of accurate joining of the broken chromosomes is not yet understood. We have studied the mechanism of reconstitution of *D. radiodurans* genome after an exposure to gamma radiation, shattering the genome down to about 25 kb fragments. We show that the highly efficient and accurate repair of fragmented *D. radiodurans* genome involves coupled DNA replication and recombination processes. A massive DNA polymerase I – dependent synthesis is required for the assembly of small chromosomal fragments into long linear intermediates, which are subsequently matured by RecA-dependent homologous recombination into functional circular chromosomes.

(RE)OBSERVATION OF DISEASES CAUSED BY DIMORPHIC FUNGI IN HUNGARY IN THE TERM OF THE PAST 50 YEARS

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Until 1955, serious dimorphic filamentous fungal infections had been unknown in Hungary and when in the next years three were detected, a discussion arose whether the maladies were indigenous, caused by autochthonous pathogens, or imported ones from known endemic areas. As no contagious cases were known, soil was suspected to be the source. Since the investigations were made by the Mycology Department, the head, Dr. Anna Csillag summarized the experience. Now, at the 50th anniversary, it seems worthwile to check the situation.

Blastomycosis (N. American or Gilchrist; agent: *Blastomyces dermatitidis)* a ubiquitous soil fungus (*cf.* the ascomycete *Emonsiella*) with some differences upon the isolation sites. “Our” sole case (first treated against tuberculosis) came from Western Hungary, near the earlier similar cases of *synov. chr. vill.* in Austria. Probably, the flu miming primary mild lung infection and the primitive thallus hinder their detection. By the revision of autopsy sections with polar light, medium-sized cells of unilateral budding with thick wall at victims with similar symptoms could be observed.

Coccidioidomycosis (desert rheumatism; agent: *Coccidioides immitis)* lives worldwide in arid deserts. All cases outside the endemic areas are considered imported ones. Near Budapest, a woman (who never left her village) was found to carry all the diagnostic symptoms of the disease and properties of the pathogen was confirmed by Prof. Vanbreuseghem (Antwerpen, Belgium). However, in the previous year, near Budapest, a rabbit kept for breeding showed the symptoms and properties of the disease and the agent has been identified. Since then, in one human case, the agent was correctly detected in direct examination upon the diagnostic spherules filled with endospores. Although a new publication reports “only” an imported case from the USA, it adds some votes to the opinion of a broader occurrence (less rigorous xerophily).

The histoplasmosis, also distributed worldwide (RES cytomycosis, cave disease; agent: *Histoplasma capsulatum* var. *capsulatum)* is again a soil fungus with affinity to pigeon guano or bat manure, whose worldwide occurrence is well known, up to the suspected role in the famous curse of Pharaoh Tuth-Ankh-Amon. In a p.m. survey of histotheca sections of 162 patients with suspected Hodgkin’s disease one real case and five dubious ones were found. Thus, in Hungary, neither in soil samples nor *in vivo* in patients was the presence of this disease found. To get indirect proofs for the presence of the disease, an intradermal histoplasmin survey was started. The survey resulted 7% positive reactions among children having an anti-Tbc. therapy, while only 1% in “normal” ones. The Tbc. like clinical picture and either the weakly characteristic saprobic thallus or its “easy” confusion with *Scopulariopsis brevicaulis* may explain the failure of direct detection. One new case was observed and accepted by detection of specific antibodies, in the Mycology Department, and reported by a European Histoplasmosis Survey in 2004.