Exploration des mécanismes impliqués dans la bioprotection d'Agaricus bisporus par les biofilms de Bacillus subtilis QST713

Caroline Pandin

université PARIS-SACLAY

NNT : 2018SACLA025

Thèse de doctorat

AgroParisTech
INSTITUT DES SCIENCES ET INDUSTRIES DU VIVANT ET DE L'ENVIRONNEMENT
PARIS INSTITUTE OF TECHNOLOGY FOR LIFE, FOOD AND ENVIRONMENTAL SCIENCES

Exploration des mécanismes impliqués dans la bioprotection d'*Agaricus bisporus* par les biofilms de *Bacillus subtilis* QST713

Thèse de doctorat de l'Université Paris-Saclay
préparée à AgroParisTech
(Institut des sciences et industries du vivant et de l'environnement)

École doctorale n°581 Agriculture, alimentation, biologie, environnement et santé (ABIES)
Spécialité de doctorat: Microbiologie

Thèse présentée et soutenue à Jouy-en-Josas, le 6 décembre 2018, par

Caroline Pandin

Composition du Jury :

M. Michael Dubow Pr. émérite, Université Paris-Saclay (I2BC) (UMR 9198)	Président
Mme Claire Prigent-Combaret DR, Université Claude Bernard Lyon 1 (UMR 5557)	Rapporteur
M. Marc Ongena Chercheur qualifié FNRS, Université de Liège	Rapporteur
M. Rémy Maufrand Responsable R&D, France Champignon	Examinateur
M. Dominique Le Coq CR, CNRS (UMR 1319)	Co-encadrant
M. Romain Briandet DR, INRA (UMR 1319)	Directeur de thèse

Remerciements

Cette thèse a été financée par le programme DIM ASTREA du conseil régional d'Ile-de-France et a été réalisée au sein de l'institut Micalis (Microbiologie de l'alimentation au service de la santé), Unité mixte de recherche (UMR1319) associant l'INRA et AgroParisTech et faisant partie de l'Université Paris-Saclay.

Je remercie tout d'abord Stéphane Aymerich, directeur de Micalis, de m'avoir accueillie au sein de son unité de recherche, accordé de son temps et prodigué de précieux conseils tout au long de ce travail de thèse.

Je remercie profondément Mme Claire Prigent-Combaret et Mr Marc Ongena d'avoir accepté d'évaluer mes travaux de thèse. J'adresse également mes remerciements à Mr Michaël Dubow et Mr Rémy Maufrand d'avoir accepté d'examiner ces travaux.

Je tiens à remercier Mr Jean-Michel Savoie, Mr Régis Védie et Mr Arnaud Bridier d'avoir participé à mes comités de thèse en apportant leurs expertises et précieux conseils.

Je remercie tout particulièrement Romain Briandet et Dominique Le Coq de m'avoir encadrée durant ces trois années, pour vos conseils, votre gentillesse et votre enthousiasme. Romain, merci de votre grande disponibilité, de votre soutient lors de ces trois années chargées en rebondissements et surtout de la confiance sans limites que vous m'avez accordée au cours de ce projet, merci aussi de votre humour parfois douteux et vos taquineries qui nous faisaient tous bien rire. Dominique, un très grand merci pour votre aide au cours de cette thèse, pour vos conseils et votre efficacité dans la relecture de mes écrits, articles et manuscript.

Un grand merci à mon binôme de bureau, Julien. Merci de ta bonne humeur, de ton humour et même du fait de t'agacer devant ton ordi, je ne suis pas la seule. Cela n'aurait pas été pareil sans toi, nos grimaces, bruitages et délires quotidiens vont me manquer.

Je remercie également les personnes m'entourant au quotidien, avec qui j'ai passé de très bons moments. Maud « le Messie », merci d'avoir pointé le bout de ton nez pour ma dernière année, un rayon de soleil dans ce ciel grisonnant d'ARN. Samia « les bons tuyaux », toujours de bonne humeur et souriante, courage ma belle c'est bientôt ton tour. Aurélia « la pile électrique », on est toutes les deux dans le même pétrin, aller dernière ligne droite, courage. Florence « le lutin rigolo » (c'est cadeau ça), une petite boule d'énergie, c'était très agréable de te taquiner au quotidien. Marina « Géo Trouvetou », ma partenaire de galère en biomol, avec tes constructions qui ne fonctionnaient pas et ma souche qui était farouche. Alexis « Sheldon », merci pour ces discussions sur tout et n'importe quoi, c'était rafraichissant. Jean-Christophe « Professeur Tournesol », ton humour borderline m'étonnait toujours et m'amusait énormément. Armelle « l'eau calme », ta quiétude m'apaisait beaucoup, merci. Merci à vous d'avoir été à mes côtés pendant cette aventure. (Pour les curieux, le petit nom que j'ai donné à Romain est « Winnie l'ourson »).

Un grand merci également à Aurélie Baliarda « marraine la bonne fée ». Merci de tes conseils, de ta joie de vivre et de ton soutient. Je remercie également Michèle Winkler pour tous ces échanges sur les ARN.

Je remercie également les personnes qui m'ont apportée leurs aides théoriques ou pratiques : Eugénie Huillet, Marion leclerc, Eliane Milohanic, Cécile Neuvéglise, Fabienne Béguet-Crespel, Camille Mayeur, Isabelle Poquet, Marie-Louise Noordine, Magali Calabre, Gwendoline Coeuret, Pascale Serror.

Merci à l'ensemble des doctorants, post-doctorants et stagiaires avec qui j'ai échangé et passé de bons moments : Rym, Célia, Elliot, Unai, Céline, Camille, Simon, Laurent, Narimane. J'en oublie probablement beaucoup, donc merci à tous.

Mes sincères remerciements vont également aux membres des plateformes MIMA2, @BriDge, GeT-PlaGe et de l'unité MaIAGE, sans qui, ce travail n'aurait pas été possible.

Je tiens à remercier également les personnes avec qui j'ai pu collaborer ou apporter mon expertise au cours de ma thèse : les membres du Centre Technique du Champignon, d'Euromycel ainsi que les membres de la Boîte à Champignon.

Merci à mes amis Lyonnais, Bourguignons et Savoyards qui m'ont soutenue durant ces trois années. En particulier un grand merci à mes amis Parisiens, qui m'ont épaulé lors de multiples épreuves personnelles parallèles à ce travail de thèse.

Merci à mon trio de cœur, Valentine, Claire et Chloé, grâce à vous je me suis épanouie et j'ai pu arriver jusqu'ici.

A ma famille, merci maman, merci papa de m'avoir toujours soutenue, fait confiance et de m'avoir laissée faire mes propres erreurs et choix, sans quoi je n'en serais pas là aujourd'hui. Merci à ma sœur d'avoir toujours été ma plus grande admiratrice et d'être si fière de moi à chaque instant, ça m'a toujours permis de repousser mes limites. Et merci pour les deux adorables cadeaux que tu m'as fait lors de cette thèse.

Et enfin, merci à toi Steph, de ton soutien, tes encouragements, ta patience lors de cette dernière année de thèse. De tes bons petits plats lorsque je ne pensais pas à me nourrir, trop concentrée à travailler.

Merci à tous !

SOMMAIRE

INTRODUCTION GENERALE

Depuis plus de 4 milliards d'années, l'évolution des espèces a été orchestrée par des processus écologiques auxquels les espèces ont dû s'adapter créant ainsi une extraordinaire biodiversité sur la planète. Cette biodiversité est fondamentale pour le fonctionnement des écosystèmes. Malheureusement, elle est en déclin depuis de nombreuses années et mise constamment en danger par les pressions que l'Homme exerce sur la nature ; notamment par la destruction des habitats causée par la conversion des milieux naturels en terres agricoles ou la déforestation, par la surexploitation des ressources naturelles comme la chasse ou la pêche, ou de la pollution. La pollution des écosystèmes est responsable d'une chute massive de la biodiversité et découle des activités de l'Homme. En effet, dans le domaine agricole, des pesticides chimiques sont utilisés depuis plus de cinquante ans afin de lutter contre les ravageurs, mauvaises herbes, maladies s'attaquant aux cultures dans un but de rendement agricole optimal. Il est clairement mis en lumière aujourd'hui que cette utilisation massive de produits chimiques affecte la résilience écosystémique des milieux naturels de par leur toxicité et leur persistance dans l'environnement.

Les microorganismes, sont des acteurs majeurs du fonctionnement biologique des écosystèmes par leurs interactions trophiques et non-trophiques, et sont particulièrement impactés par la présence de pesticides. Initié en 2008 en France, le plan EcoPhyto I mis en place par le gouvernement visait à réduire de 50% l'utilisation des pesticides chimiques dans un délai de 10 ans. En 2018, cet objectif est très loin d'être atteint. L'ambition du plan a été actualisée avec un programme progressif plus en phase avec les avancées scientifiques du domaine : le plan EcoPhyto II. Ce nouveau plan d'action vise une réduction de 25% de l'utilisation de ces produits phytosanitaires chimiques à l'horizon 2020, puis une réduction de 50% à l'horizon 2025. Cet objectif repose notamment sur un large déploiement de méthodes de lutte biologique utilisant des organismes vivants naturels (macroorganismes, microorganismes…) afin de gérer les populations de bioagresseurs. Dans le cas de maladies culturales d'origines microbiennes, les mécanismes d'action exercés par les agents de biocontrôle microbiens sont encore peu décrits. Ils concernent principalement l'antagonisme des flores pathogènes par la sécrétion de molécules antimicrobiennes, les phénomènes de compétition (spatiale et nutritionnelle) ou encore la stimulation de la croissance des plantes et de leurs défenses naturelles.

Dans ce projet, nous avons voulu intégrer la notion de « biofilm » dans ces mécanismes de bioprotection des cultures. Le mode de vie en biofilm est le mode de vie préférentiel des microorganismes dans leurs environnements naturels. Les cellules spatialement organisées en biofilm sont dotées d'une physiologie particulière leur permettant de faire face aux divers stress environnementaux auxquels elles sont soumises. Les cellules regroupées en communautés sous forme de biofilm possèdent des capacités de tolérance à l'action des composés toxiques et de persistance accrues en comparaison avec leurs homologues planctoniques. Ces propriétés de tolérance de communautés microbiennes spatialement organisées sont telles que le NIH (National Institute for Health) aux Etats-Unis a publié une étude suggérant qu'environ 80% des infections humaines seraient liées à la formation de biofilms. La formation de ces structures biologiques est si importante en santé humaine que nos collègues anglo-saxons du domaine de la santé utilisent aujourd'hui le terme de « Biofilm-Associated Infections » dans le cas de certaines infections chroniques. Les recherches intensives réalisées depuis 30 ans ont également montré une physiologie particulière de ces communautés de surface, avec dans certains cas des modulations importantes dans la synthèse de métabolites. La synthèse de certains antimicrobiens peut être amplifiée ou conditionnée à ce mode de vie social. Alors que les capacités de survie, de compétition et d'inhibition des pathogènes culturaux par les agents de biocontrôle dépendent très probablement (comme dans le domaine de la santé) de cette organisation en biofilm, très

peu d'études scientifiques ont intégré ce mode de vie dans la compréhension de l'efficacité des traitements de bioprotection.

Nous nous sommes intéressés à une filière singulière Française, celle des « champignons de Paris », qui utilise un produit de biocontrôle à base d'une bactérie « *Bacillus subtilis* » dans près de 80% de la production afin de lutter contre une moisissure les décimant, *Trichoderma aggressivum*. Plus de 50 exploitations agricoles, majoritairement situées dans le Maine-et-Loire et en Région Parisienne, assurent la production d'*Agaricus bisporus* connu sous les noms de champignon de couche ou champignon de Paris. La coopérative « France Champignons » rachetée en 2010 par le groupe Bonduelle produit la majeure partie des champignons Français. La culture de ce champignon ne se réalise pas en plein champs, et plus ou très peu en caves comme c'était le cas au début du siècle. La culture se réalise aujourd'hui principalement en hangars climatiques, dans des conditions environnementales très contrôlées (température, humidité…). Ce contrôle des conditions de culture (contrairement aux cultures en plein champs soumises en particulier aux fluctuations climatiques) en fait un modèle de choix pour l'étude des mécanismes de bioprotection de son substrat de culture, le compost.

C'est dans ce contexte que s'inscrit mon travail de thèse qui a consisté à étudier les mécanismes d'action du seul agent de biocontrôle disposant d'une autorisation de mise sur le marché dans cette filière (au démarrage du projet) : la souche *Bacillus subtilis* QST713, agent actif de la gamme commerciale Serenade ®. Ce projet de thèse a bénéficié d'un financement de la Région Ile-de-France via son dispositif de soutien à la recherche DIM ASTREA (domaines d'intérêts majeurs - agrosciences, écologie des territoires, alimentation). Ces travaux ont été réalisés au sein de l'Institut Micalis (unité mixte de recherche associant l'Institut National de la Recherche Agronomique (INRA) et AgroParisTech, site de Jouy-en-Josas, Yvelines) dans les équipes B2HM (bioadhésion-biofilm et hygiène des matériaux) et GCBS (contrôle génétique dans les systèmes bactériens). Ces travaux n'auraient pas été possibles sans l'appui des professionnels de la filière, et en particulier l'étroite collaboration expérimentale avec le CTC (Centre Technique du Champignon) basé à Distré (Maine-et-Loire).

Ce manuscrit se décline en trois parties principales.

La première partie correspond à une synthèse bibliographique présentant le contexte de ce travail. Le chapitre 1 présente un état de l'art sur les trois piliers de ce système : *A. bisporus* et son mode de culture ; un des pathogènes majeurs décimant les cultures, *T. aggressivum* ; le biocontrôle et l'agent de biocontrôle utilisé dans la filière. Le chapitre 2 présente un état de l'art sur la formation de biofilms par les microorganismes, l'avantage de leur physiologie particulière et leur intérêt dans le cadre de la protection des cultures.

La seconde partie rassemble les résultats obtenus lors de ce travail. Dans le premier chapitre, afin de décrypter les mécanismes d'action de l'agent de biocontrôle utilisé dans la filière des champignons de couche, nous avons entrepris de séquencer le génome de la souche QST713 afin d'explorer ses spécificités génomiques reliées à la formation de biofilm et la production de molécules antimicrobiennes et en les comparant à d'autres souches de la même espèce utilisées en biocontrôle. Dans le second chapitre, nous nous sommes ensuite intéressés à l'évolution des communautés microbiennes du compost lors de la culture d'*A. bisporus*, en présence ou non de l'agent de biocontrôle et/ou de *T. aggressivum* afin de déterminer l'impact écologique de l'addition de cet agent dans les cultures. Dans le dernier

chapitre de résultats et dans le but d'enrichir notre connaissance sur une partie des mécanismes de biocontrôle potentiellement effectifs dans ce système, nous avons développé un micro-modèle de culture simplifiée à trois partenaires en enceinte climatique de laboratoire. Ce système nous a permis de déterminer l'implication relative de la formation de biofilm et de la synthèse d'antimicrobiens dans ces mécanismes, et d'étudier la reprogrammation cellulaire de l'agent de biocontrôle dans des conditions aussi proches que possibles que celles des hangars climatiques utilisés industriellement.

Ce manuscrit se clôture dans une troisième partie par une discussion générale mettant en perspective l'ensemble des résultats obtenus et les voies de recherches ouvertes dans le contexte du biocontrôle microbien.

SYNTHESE BIBLIOGRAPHIQUE

Chapitre 1: Le pathosystème *Agaricus bisporus* - *Trichoderma aggressivum* f. *europaeum* – agent de biocontrôle

1.1 Le modèle champignon de couche, *Agaricus bisporus*

1.1.1 Histoire

Les plus anciennes traces du champignon de couche remonteraient à l'Egypte antique, des archéologues auraient identifiés un *Agaricus* peints sur les murs du tombeau d'un Pharaon datant de 1450 av. J.-C, mais l'histoire du champignon de Paris reste incertaine (Savoie and Mata, 2015). A partir de l'an 600 av. J.-C, il aurait été cultivé en Asie, et par les grecs un peu plus récemment.

En France, ce n'est qu'à partir du XVII^{ème} siècle sous le règne de Louis XIV où Jean-Baptiste La Quintinie, agronome et jardinier du roi, cultivait le champignon de couche dans les jardins de Versailles en plein air au printemps et en automne. La méthode de culture du champignon aurait été décrite par trois botanistes, Olivier de Serres en 1600, Mardhault en 1678 et Piton de Tournefort en 1707 mais restait une énigme pour l'époque.

« Adrien Tournebus s'expliquait la production de champignons comme celle de bulles d'eau qui se forment lorsque le liquide tombe sur un fer chaud ... De là naquit l'usage d'enfermer du fumier chaud dans la terre pour échauffer celle-ci, puis de l'arroser avec de l'eau froide afin de lui faire produire des bulles »

Victor Paquet, Traité de la culture de champignons, 1847

A la fin du XVIII^{ème} siècle, sous l'époque de Napoléon Ier, la culture du champignon apparait dans les carrières souterraines de Paris. Différentes anecdotes sur les débuts de cette culture en carrière sont relatées et exposées dans ces trois références internet suivantes (Hubert, n.d.; Les champignonnières, n.d.; Nicolas, n.d.). L'une d'entre elles explique qu'un agriculteur « Chambry », du village de Passy, après des essais infructueux de cultures dans son jardin, jeta le fumier qu'il utilisait dans des carrières abandonnées. Il revint quelques mois plus tard et découvrit que les champignons s'étaient développés et décida de lancer son exploitation de « champignons de Passy », pour les différencier de ceux cultivés en plein air. Cette culture en carrière se répandit en banlieue parisienne et le nom « champignons de Paris » fît son apparition.

Du XIX^{ème} siècle au début du XX^{ème} siècle, les projets de construction du métro parisien débutèrent et la culture des champignons dut se déplacer. Elle se répandit dans plusieurs régions dont le Val de Loire, vers Saumur avec la reconversion de ses nombreuses carrières en champignonnières.

Aujourd'hui, ce mode de culture en caves et carrières reste rare et les champignons de Paris sont cultivés à 80% en hangars climatiques (Source : CTC, Centre Technique du Champignon, Distré) et majoritairement dans le département du Maine-et-Loire à Doué-la-Fontaine à côté de Saumur.

Depuis la nouvelle réglementation européenne (CE) n°982/2002, la dénomination officielle du champignon de Paris est champignon de couche afin éviter les conflits sur l'origine régionale du produit.

1.1.2 Production mondiale et européenne

La production mondiale de champignons toutes variétés et truffes en 2016 se chiffre à 11 millions de tonnes (Fig. 1). La Chine se place en première position sur ce marché et représente 72% de cette production. La France se classe en 9ème position avec 0,94% (101949 tonnes) du marché mondial de production de champignons toutes variétés et truffes derrière la Chine, l'Italie, les États-Unis, les Pays-Bas, la Pologne, l'Espagne, l'Iran et le Canada (Source : Food and Agriculture Organization, FAOSTAT 2017).

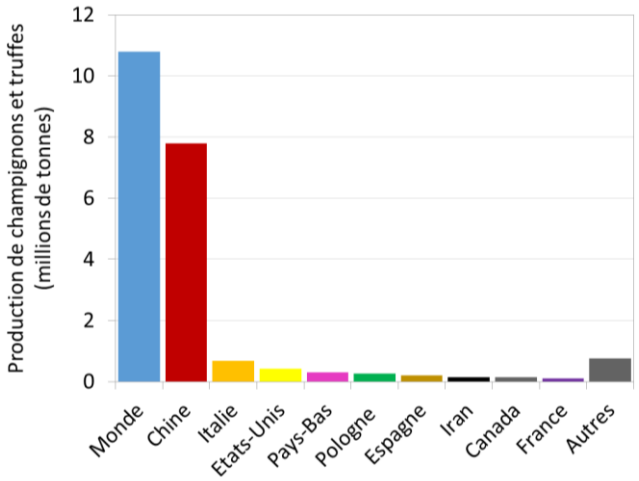

Fig. 1: Production mondiale de champignons et truffes en 2016 (Source : FAOSTAT 2017).

Le champignon de couche (*A. bisporus*) représente environ 40% de cette production mondiale. Au niveau européen, les quatre producteurs majeurs d'*A. bisporus* sont les Pays-Bas, la Pologne, la France et l'Espagne (FranceAgriMer and ANICC, 2015; Royse et al., 2017). Ces vingt dernières années, l'évolution de la production d'*A. bisporus* a considérablement changé entre ces quatre producteurs. Les Pays-Bas, la Pologne ainsi que l'Espagne étaient en constante augmentation de 1996 à 2012 (Fig. 2). Les Pays-Bas ont connu une augmentation de 20% de leur production jusqu'à atteindre 296000 tonnes de champignons en 2012, 42% pour l'Espagne (100000 t en 2012) et 68% pour la Pologne (255000 t en 2012). *A contrario*, la France a connu une diminution progressive (35%) de sa production jusqu'à atteindre 107500 tonnes de champignons de couche produits en 2012.

Cette diminution de production est la conséquence de plusieurs facteurs, d'une part, l'augmentation des importations due à la consommation grandissante des champignons de couche par les français (ANICC, 2014), et d'autre part, la diminution des exportations induite par la forte compétitivité des pays européens. Cette perte de compétitivité est principalement due au coût élevé de la main d'œuvre en France comparé au pays européens et notamment face à la Pologne. En 2012, la France se positionnait en 3ème place sur le marché européen. Cependant, un récent rapport sur le suivi concurrentiel de production du champignon de couche en Europe en 2016 a révélé le descente de la France à la 4ème place et la montée de l'Espagne à la 3ème position (FranceAgriMer and ANICC, 2017).

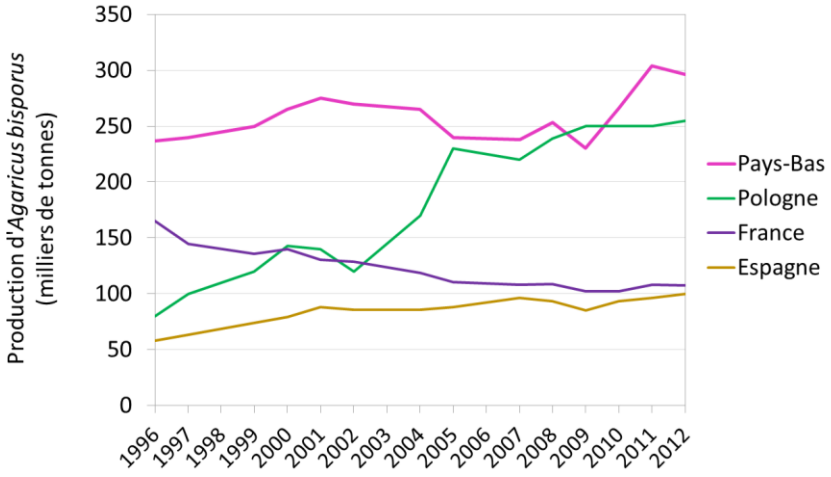

Fig. 2: Evolution de la production d'*Agaricus bisporus* des 4 principaux producteurs Européens de 1996 à 2012 (FranceAgriMer and ANICC, 2015).

1.1.3 Mode de culture d'*Agaricus bisporus*

Agaricus bisporus est un champignon basidiomycète (Règne : Fungi ; Phylum : Basidiomycota ; Classe : Agaricomycetes ; Ordre ; Agaricales ; Famille : *Agaricaceae* ; Genre : *Agaricus* ; Espèce : *bisporus*) caractérisé par ses organes de reproduction, les basides, situées dans les lamelles du sporophore (Fig. 3). Ce sont les basides qui produisent les spores du champignon. Une fois relâchées, ces spores germent et donnent naissance au mycélium, lequel va proliférer dans l'environnement. Lorsque les conditions environnementales deviennent difficiles pour cette prolifération, le mycélium enclenche un processus de fructification puis va former des corps fructifères passant par plusieurs stades de développement ; (i) les « têtes d'épingles » qui sont de petites sphères de deux millimètres de diamètre, (ii) les primordia, jeunes sporophores en formation (0,5 – 1 cm de diamètre), puis (iii) les champignons matures caractérisés par un pied (ou stipe) et un chapeau (carpophore ou sporophore) qui abrite les lamelles arborant les basides. C'est sous ce dernier stade que le champignon va être capable de produire des spores qui lui permettront à la fois de survivre aux conditions environnementales difficiles, et d'être disséminé grâce aux éléments naturels (vent, …).

Ce champignon est très peu retrouvé à l'état sauvage. Son mode de culture en cave, bien qu'il soit encore pratiqué reste relativement rare aujourd'hui et a été remplacé par une culture industrielle en hangars climatiques sous conditions contrôlées (Fig. 4). Sa culture est précédée d'une phase de préparation du substrat de culture qui dure environ un mois. Ce substrat de culture est préparé principalement à partir de fumier de cheval et de paille de blé soumis à un processus de compostage (humification) permettant la conversion des matières organiques en un produit stabilisé riche en minéraux et composés humiques. Le compost est ensuite « Pasteurisé » une semaine en tunnel avant utilisation en culture afin de diminuer la présence potentielle de flores pathogènes d'*A. bisporus*.

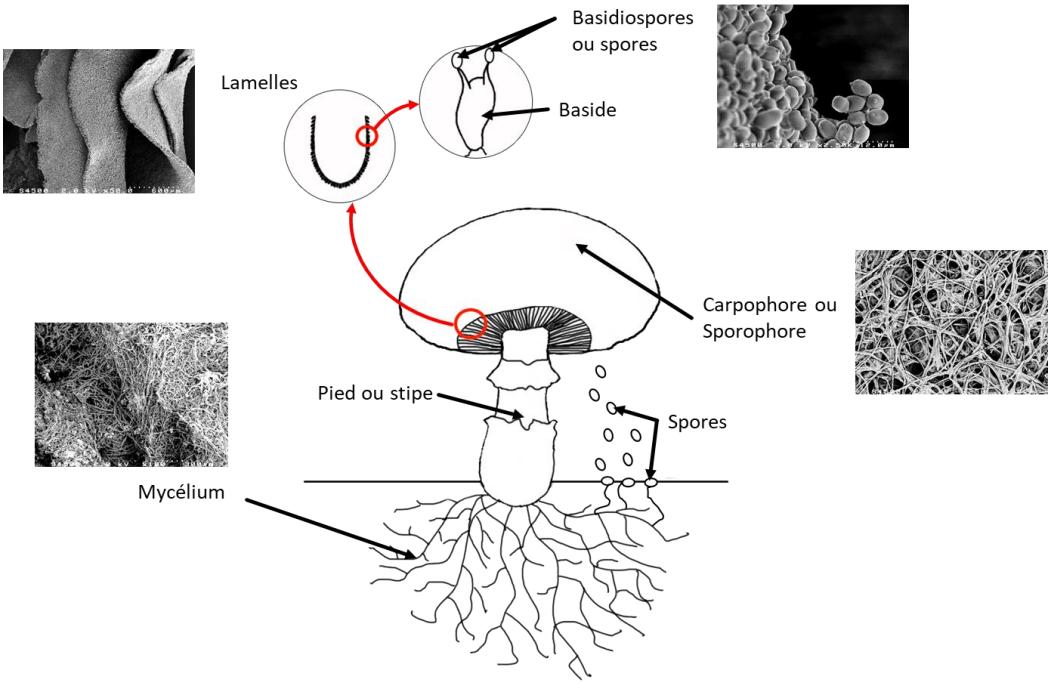

Fig. 3: Schéma simplifié du cycle de vie d'*Agaricus bisporus*. Visualisation par microscopie électronique à balayage de mycélium d'*A. bisporus* dans le compost, de lamelles, de spores et du sporophore d'*A. bisporus*.

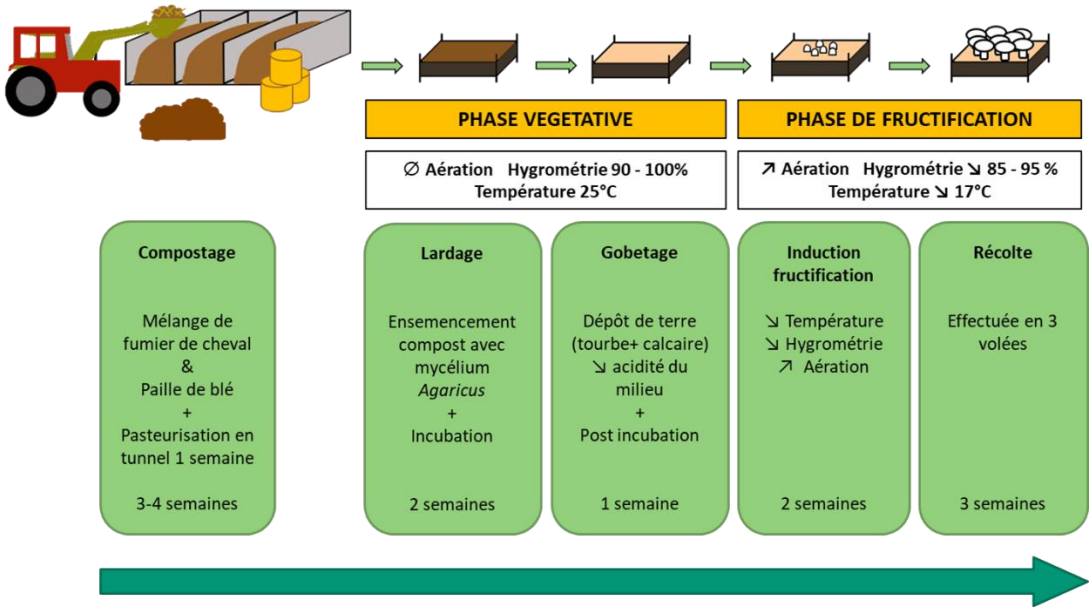

Fig. 4: Mode de culture d'*Agaricus bisporus*. Cycle total de trois mois comprenant la préparation du substrat de culture (Adapté d'après les informations collectées auprès de la société France Champignon).

La culture d'*A. bisporus* se déroule en deux phases d'une durée totale de deux mois (Fig. 4). La première est la phase végétative qui permet l'envahissement du compost par le mycélium de l'agaric. Cette phase se découpe en plusieurs étapes. Le lardage, qui consiste à ensemencer le compost avec le mycélium d'*A. bisporus*. La semence utilisée est formulée sous forme de grains d'orge sur lesquels le mycélium d'*A. bisporus* s'est développé (Fig. 5A). Dans la filière industrialisée des champignons en France, c'est la variété *Agaricus bisporus* var. *bisporus* qui est utilisée (Amycel Delta, Amycel, Vendôme). Le compost ensemencé est ensuite incubé en salles de culture (Fig. 5B) pendant deux semaines sous conditions contrôlées (température : 25°C ; hygrométrie : 90 - 100% ; concentration en CO_2 supérieure à 0,1%). En fin d'incubation, lorsque le mycélium d'*A. bisporus* a envahi le compost, l'étape de gobetage est effectuée (Fig. 6). Le gobetage consiste à déposer une terre sur le compost qui est ensuite incubé une semaine supplémentaire et permet la colonisation de la terre de gobetage par le mycelium d'*A. bisporus*, c'est l'étape de post-incubation. Cette terre est composée de tourbe et de calcaire et permet de neutraliser l'acidité du milieu, et par ce biais de favoriser la fructification lors de la phase suivante en appliquant un stress physico-chimique. La seconde phase de la culture est la phase de fructification permettant le développement du carpophore comestible du champignon et sa récolte afin d'être commercialisé. Cette phase consiste à induire la fructification du champignon en appliquant un stress thermique par une diminution de la température de 25°C à 17°C ; une baisse de l'hygrométrie à 85 - 95% et une augmentation de la ventilation avec un taux de CO_2 inférieur à 0,1% sont également appliquées. Un inducteur biologique interviendrait également dans la fructification : la terre de gobetage apporte une quantité massive de *Pseudomonas* qui, en stressant le champignon, stimuleraient la fructification d'*A. bisporus* (Rainey, 1991; Singh et al., 2013; Colauto et al., 2016).

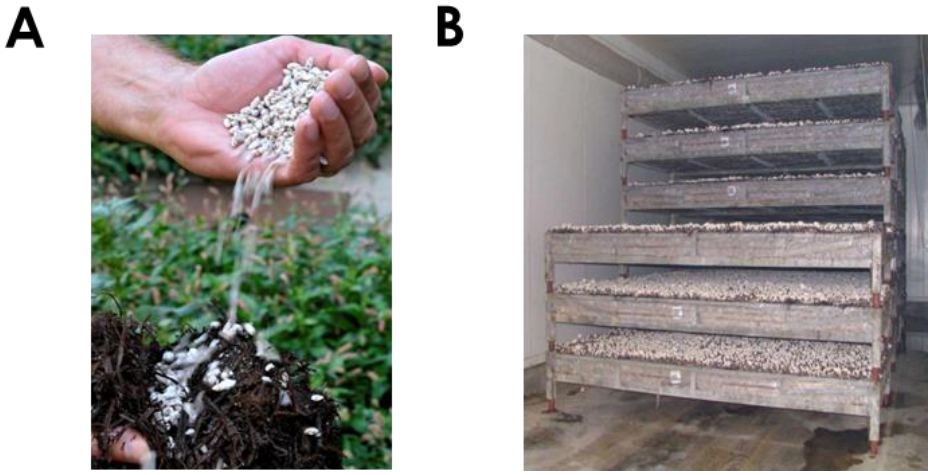

Fig. 5: Différentes formes d'*Agaricus bisporus*. (A) Semence d'*Agaricus bisporus* développé sur grains d'orge, (B) fructification en salle de culture (Source : France Champignon).

Les premiers champignons matures peuvent être récoltés une dizaine de jours après l'induction. La fructification se déroule en « volées », caractérisées par l'apparition synchrone de champignons matures. Une dizaine de volées espacées d'une semaine peuvent être obtenues lors d'un cycle complet de culture mais d'abondance moindre pour les dernières, dans la filière française, trois volées et donc récoltes sont réalisées pour une meilleure rentabilité.

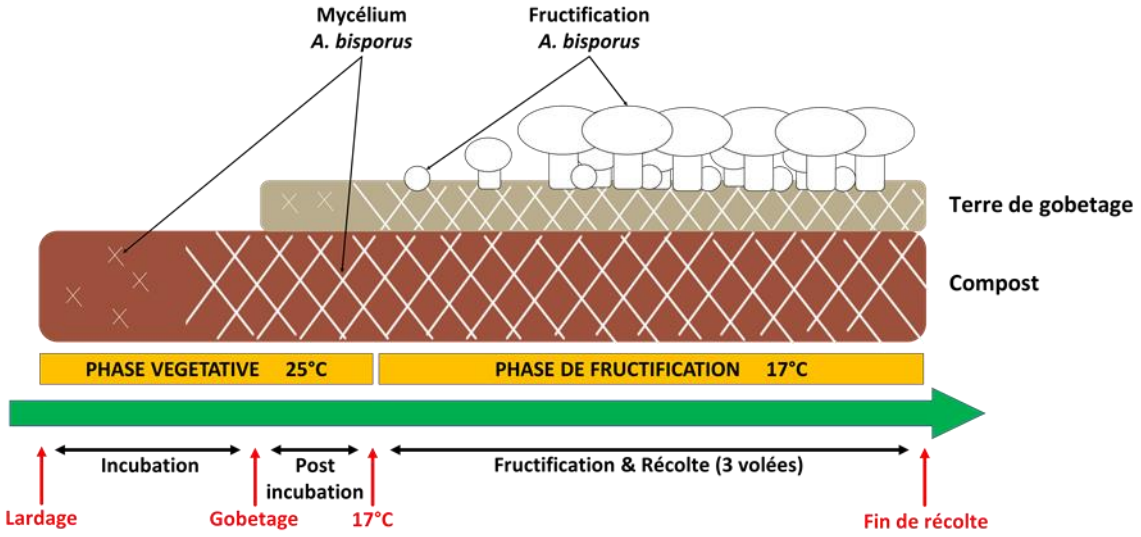

Fig. 6: Représentation schématique de la culture d'*Agaricus bisporus* au cours du temps.

1.1.3.1 Un équilibre fragile : flores indésirables intervenant lors de la culture

Agaricus bisporus est soumis à différentes attaques de flores indésirables lors de son développement mycélien ainsi que lors de sa fructification (Fletcher and Gaze, 2008; Largeteau and Savoie, 2010). Lors de la phase végétative, des flores compétitrices telles que les moisissures *Chaetomium olivaceum* (Olive-green mould), *Scopulariospsis fimicola* (plaster mould) affectent la croissance mycélienne d'*A. bisporus*, (Largeteau and Savoie, 2010). Un des pathogènes majeurs d'*Agaricus bisporus* est la moisissure *Trichoderma aggressivum* (green mould disease), puisqu'elle empêche le développement de celui-ci et affecte grandement le rendement en champignons (Samuels et al., 2002). Le paragraphe suivant est dédié aux spécificités de cette moisissure indésirable au vu de son importance économique pour la filière. Lors de la phase de fructification, différentes maladies sont susceptibles de toucher *A. bisporus* et d'altérer le développement du carpophore. De nombreux *Pseudomonas* sont responsables d'une coloration du sporophore ; l'apparition de taches de couleur brune (brown blotch disease) sont causées par *P. tolaasii* (Grewal and Rainey, 1991), *P. protegens* (Henkels et al., 2014), *P. reactans* (Abou-Zeid, 2012), *P. constantinii* (Munsch et al., 2002) ou *P. syringae* (Godfrey et al., 2001). Des taches de couleur jaune (ginger blotch) ont été associées à la présence de *P. gingeri* (Godfrey et al., 2001). D'autres espèces de *Pseudomonas* sont capables d'assécher ou au contraire de rendre suintant le champignon (Godfrey et al., 2001; Largeteau and Savoie, 2010). Comme mentionné précédemment, les *Pseudomonas* seraient des inducteurs importants du processus de fructification d'*A. bisporus* ce qui rend difficile le traitement de ces maladies sans affecter le rendement en champignon. Le champignon de couche peut également être affecté par des moisissures lors de cette phase, notamment *Lecanicillium fungicola* (anciennement *Verticillium fungicola*) responsable de la môle sèche (dry bubble disease) induisant une déformation du champignon (Largeteau and Savoie, 2008; Siwulski et al., 2011; Berendsen et al., 2012) ou *Mycogone perniciosa* responsable de la môle humide (wet buble disease) (Glamočlija et al., 2008; Siwulski et al., 2011). Autant de maladies dont le traitement devra être réalisé sans fongicides chimiques dans les années à venir.

1.2 La maladie de la moisissure verte, *Trichoderma aggressivum*

Trichoderma spp. est un champignon ascomycète (Règne : Fungi ; Phylum : Ascomycota ; Classe : Sordariomycetes ; Ordre ; Hypocreales ; Famille : *Hypocreaceae* ; Genre : *Trichoderma*) ubiquitaire et notamment présent dans les sols et en association avec les plantes (Harman et al., 2004). La morphologie des différentes espèces de *Trichoderma* est très proche. Ils ont une reproduction asexuée, les spores (conidies) donnent naissance après germination à un mycélium blanc, fin et septé, disposant de nombreuses ramification portant les phialides en forme de quilles (Fig. 7A) (Samuels et al., 1994; Samuels, 1996). Ce n'est qu'après plusieurs jours que les spores vont être produites par conidiogénèse et donner cette coloration verte typique à la culture (Fig. 7B).

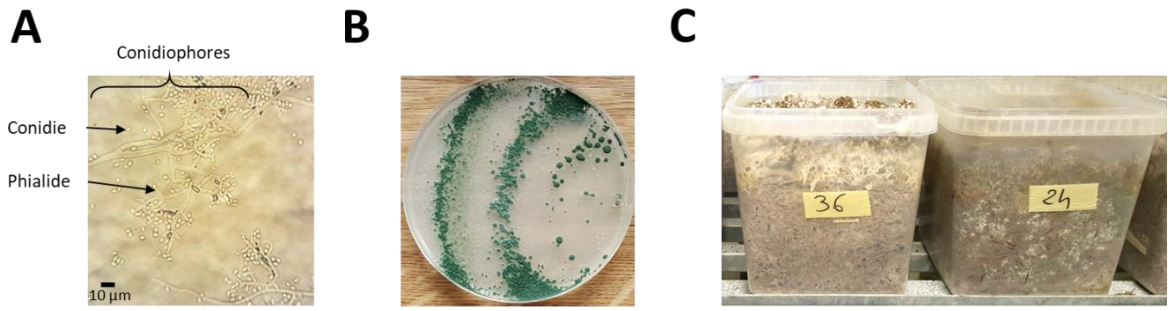

Figure 7: Aspects morphologiques de *T. aggressivum* f. *europaeum* (Ta2). (A) Etat frais d'une culture de *T. aggressivum* incubée 15 jours à 25°C sur gélose à l'extrait de malt. (B) Culture de *T. aggressivum* sur gélose à l'extrait de malt incubée 15 jours à 25°C, visualisation de la sporulation verte. (C) Pots de culture d'*A. bisporus* en compost à 28 jours, (36) Developpement mycélien d'*A. bisporus*, (24) *A. bisporus* contaminé par *T. aggressivum*, apparition de la sporulation verte de *T. aggressivum*.

De nombreuses espèces de *Trichoderma* telles que *T. harzianum* et *T. viride* sont utilisées dans les cultures agricoles afin de protéger les plantes contre leurs pathogènes fongiques (Harman, 2006). Leurs mécanismes d'action seraient le mycoparasitisme des pathogènes fongiques, la compétition spatiale et nutritive, la sécrétion d'antimicrobiens ainsi qu'une amélioration de la croissance des plantes et l'induction de leur système de défenses (Williams et al., 2003; Harman et al., 2004; Harman, 2006). Ils possèdent également une batterie d'enzymes permettant la dégradation des parois cellulaires des pathogènes de plantes (Viterbo et al., 2002). Dans d'autres environnements, *T. viride* est responsable de maladies chez le concombre et la tomate (Menzies, 1993). De la même façon, certaines souches de *T. harzianum* sont responsables de la maladie de la moisissure verte dans les cultures d'*A. bisporus*. Les biotypes *T. aggressivum* f. *europaeum* (Ta2) retrouvé en Europe et *T. aggressivum* f. *aggressivum* (Ta4) retrouvé dans le nord de l'Amérique étaient anciennement nommées *T. harzanium* Th2 et *T. harzianum* Th4 respectivement (Ospina-Giraldo et al., 1998; Hermosa et al., 1999; Samuels et al., 2002). C'est le caractère « agressif » de ces deux biotypes dans les cultures qui leur a valu ce changement de nom en comparaison aux biotypes « non-agressifs », *T. harzanium* Th1 et Th3. Ces deux biotypes Ta2 et Ta4 déciment les cultures d'*A. bisporus* depuis la fin des années 1980, induisant des pertes massives de production du champignon de couche (Seaby, 1996). La croissance mycélienne d'*A. bisporus in vitro* serait inhibée par un composé antifongique produit seulement par les biotypes « agressifs », le 3,4-dihydro-8-hydroxy-3-methylisocoumarin. Cependant ce composé n'a pas été retrouvé dans le compost

contaminé par le biotype Ta4, probablement dégradé par la microflore du compost productrice de lactonase (Dong et al., 2001; Krupke et al., 2003). La croissance d'*A. bisporus* et *T. aggressivum* Ta2 serait concomitante jusqu'à ce que l'espace du milieu soit entièrement colonisé (Mumpuni et al., 1998). Le contact entre les deux mycéliums déclencheraient la sporulation de *T. aggressivum* qui inhiberait la croissance d'*A. bisporus* (Mamoun et al., 2000). De plus, *A. bisporus* produirait des métabolites inhibant la croissance des biotypes « non-agressifs » mais stimulant la croissance du biotype Th2 (Mumpuni et al., 1998). Lors de la culture d'*A. bisporus*, la colonisation visible du compost par *T. aggressivum* se traduit en fin de phase d'incubation par l'apparition d'une couleur verte (Fig. 7C), reflet de la sporulation de la moisissure. Avant ce stade, il est difficile de distinguer visuellement la contamination par *T. aggressivum* du développement normal du mycélium du champignon de couche. Chez *T. aggressivum*, les gènes, *prb1* codant pour une protéinase, *ech42* codant une endochitinase et un gène codant pour une β-glucanase sont surexprimés durant l'interaction *A. bisporus* - *T. aggressivum,* indiquant la possible implication de ces enzymes de dégradation de la paroi cellulaire dans le mycoparasitisme par *T. aggressivum* (Guthrie and Castle, 2006; Abubaker et al., 2013). La dégradation par ces enzymes de la paille de blé du compost ainsi que de la microflore fournit une source de nutriments à *Trichoderma*. Une étude de 2003 a émis l'hypothèse que l'agressivité des biotypes Ta2 et Ta4 seraient un phénomène découlant de leur croissance massive due à la dégradation de la paille de blé (Williams et al., 2003).

1.3 Le biocontrôle ou comment utiliser des mécanismes naturels de protection des cultures

1.3.1 Des objectifs gouvernementaux ambitieux

Dans le monde, les pertes de rendements agricoles dues aux pathogènes, ravageurs et mauvaises herbes représentent 20 à 40% de la production agricole (Teng and Krupa, 1980; Teng, 1987; Oerke, 2006; Savary et al., 2012). Dans un contexte d'après-guerre, la course au rendement et au profit a favorisé l'utilisation de pesticides chimiques, une solution facile à mettre en œuvre et radicale afin de protéger les cultures. Quelques décennies plus tard, il ne fait plus de doute que cette utilisation massive de pesticides a engendré une pollution environnementale chimique alarmante ainsi qu'un impact néfaste sur les écosystèmes naturels (Di Poi et al., 2017; Uwizeyimana et al., 2017; Lu and Lu, 2018; Otalvaro and Brigante, 2018; Perugini et al., 2018). Ironiquement, l'utilisation massive de ces pesticides chimiques, dans le but d'améliorer les rendements agricoles, a conduit à la progressive destruction des ressources naturelles, dont la biodiversité microbienne des sols qui, a un rôle majeur dans la productivité des plantes et le contrôle des maladies (Barrios, 2007; Luan et al., 2015; Wu et al., 2015).

Aujourd'hui en France, afin de palier à ce problème majeur, le gouvernement a mis en place le plan « Ecophyto 2 » visant à réduire de 50% l'utilisation des pesticides à l'horizon 2025, notamment par l'utilisation du biocontrôle (Ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt and Ministère de l'Ecologie, du Developpement durable et de l'Energie, 2016). D'après le ministère de l'Agriculture, **le biocontrôle est un ensemble de méthodes de protection des cultures basé sur l'utilisation de substances naturelles ou d'organismes vivants naturels visant à gérer les populations de bio-agresseurs plutôt qu'une totale éradication**. Le fondement de ces techniques se base sur les mécanismes et interactions qui régissent les relations entre espèces au sein du milieu naturel. Il existe quatre familles de biocontrôle : (i) les macroorganismes auxiliaires comme les invertébrés, insectes, acariens, nématodes dans le but d'attaquer les bio-agresseurs (e. g. coccinelles utilisées contre les

pucerons dans les systèmes viticoles) (ii) les microorganismes tels que les bactéries, champignons et virus permettant de contrer les maladies dues aux pathogènes de plantes et de stimuler les défenses naturelles des plantes, (iii) les médiateurs chimiques comme les phéromones et kairomones basés sur les méthodes de confusion sexuelle et piégeage (e. g. permet l'éloignement des systèmes viticoles des papillons, dont les larves dévorent les feuilles et affaiblissent la plante) et (iiii) les substances naturelles d'origine végétale, animale ou minérale présentes naturellement dans le milieu.

D'après un communiqué de presse d'IBMA France (International Biocontrol Manufacturers Association), le biocontrôle ne représente aujourd'hui que 5% de la protection des cultures en France. Les substances naturelles sont la méthode de bioprotection la plus utilisée en 2016 en France avec 57% des ventes de produits de biocontrôle, devant les médiateurs chimiques, les macroorganismes et les microorganismes (Fig. 7) (IBMA France, 2017).

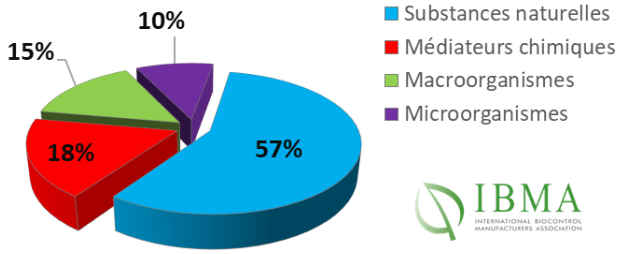

Fig. 8: Vente de produits de biocontrôle en France en 2016 (IBMA France, 2017).

Depuis une dizaine d'années en France, la filière des champignons de couche s'est singularisée par une utilisation massive du biocontrôle qui protège près de 80% des cultures par un agent de biocontrôle bactérien (communication personnelle, Centre Technique du Champignon (CTC)).

1.3.2 Le biocontrôle dans la filière des champignons de couche

Dans la filière des champignons de couche, 25% des pertes annuelles sont dues à des altérations microbiologiques dont 5 à 10 % causées par *T. aggressivum* (Soković and Van Griensven, 2006). Depuis 2008, la filière s'est tournée vers une approche de biocontrôle et utilise le produit de biocontrôle « Sérénade ® », un biofungicide utilisé pour le traitement du compost de culture d'*A. bisporus* contre *T. aggressivum* et formulé à base de spores de la bactérie, *Bacillus subtilis* QST713.

Le centre technique du champignon (CTC) effectue régulièrement des campagnes d'essais de biocontrôle afin de vérifier la stabilité de l'effet du produit Sérénade ® sur le rendement en champignons en présence ou non de *T. aggressivum*. Les données de rendement en champignon de ces études en 2013 et 2014 ont été recueillies et analysées. En présence du pathogène *T. aggressivum*, une diminution du rendement en champignon de 52% en moyenne a été observée à la dose inoculée (*T. aggressivum* : 200 spores) (Fig. 8A). Ces données montrent l'impact néfaste de cette moisissure sur les cultures se

traduisant par l'apparition d'une zone verte de sporulation en fin de phase végétative et une absence de fructification d'*A. bisporus* en fin de phase de fructification (Fig. 8B). Lorsque le compost de culture inoculé avec *T. aggressivum* était traité avec l'agent de biocontrôle (BCA) *B. subtilis* QST713, un retour à un rendement équivalent au rendement témoin était observé (Fig. 8A), avec une absence de zone verte et une fructification d'*A. bisporus* effective (Fig. 8B), montrant une nette efficacité du traitement.

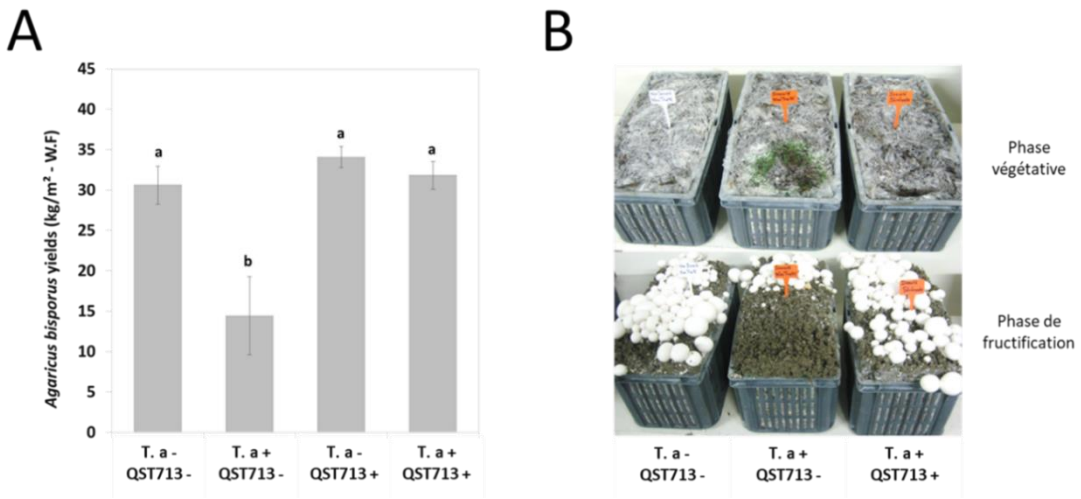

Fig. 9: Effet biocontrôle de *B. subtilis* QST713 utilisé contre *T. aggressivum*. (A) Données de rendement en champignons en 2013 - 2014 au CTC en kilogramme par mètre carré de pieds entiers (W. F.). Les données représentées avec une lettre similaire ne sont pas significativement différentes. (B) Photos des pots de cultures en fin de phase végétative (haut) et fin de phase de fructification (bas). Pots traités avec *B. subtilis* QST713 (QST713 +) ou non (QST713 -) et inoculés avec *T. aggressivum* (T. a +) ou non (T. a -). Invasion par *T. aggressivum* : zone de sporulation verte (Source CTC).

À l'heure actuelle, les mécanismes de bioprotection par les microorganismes restent encore peu connus à ce jour, ils impliqueraient la compétition nutritionnelle, la stimulation des défenses des plantes et la production de molécules antimicrobiennes (Whipps, 2001; Ongena et al., 2005; Ongena and Jacques, 2008; Cawoy et al., 2015; Saravanakumar et al., 2016).

1.3.2.1 L'agent de biocontrôle *Bacillus subtilis* QST713

Bacillus subtilis est une bactérie sporulante ubiquitaire retrouvée dans de nombreuses niches écologiques incluant les sols. Elle fait partie du « *Bacillus subtilis* species complex ». Avant la généralisation des techniques de séquençage et le moindre coût de celles-ci, la taxonomie des espèces de ce complexe restait difficile et ne permettait pas une classification claire et stable (Gordon et al., 1973; Fritze, 2004; Rooney et al., 2009). Une étude de Gordon et al. (1973) présentait une classification composée de quatre clades : Le clade I « *B. subtilis* », regroupant les espèces *B. subtilis* subsp. *subtilis*, *B. subtilis* subsp. *spizizenii*, *B. vallismortis*, *B. clausii*, *B. mojavensis*, *B. atrophaeus*, *B. amyloliquefaciens* ; le clade II « *B. licheniformis* », regroupant les espèces *B. licheniformis* et *B. sonorensis* ; le clade III « *B. pumilus* » composé exclusivement des *B. pumilus* ; le clade IV « *B. firmus* », composé de *B. firmus* et *B. lentus*.

Ces trois dernières années, grâce à l'augmentation de la disponibilité des nombreuses séquences génomiques de souches de ce complexe, le « *Bacillus subtilis* species complex » a subi une grande reclassification basée sur des analyses phylogénomiques et bioinformatiques (Teeling et al., 2004; Konstantinidis and Tiedje, 2005; Richter and Rossello-Mora, 2009; Meier-Kolthoff et al., 2013; Richter et al., 2015; Dunlap et al., 2015, 2016; Fan et al., 2017). Ces nouvelles analyses ont donné naissance à une nouvelle classification exposée dans la Table 1 (Fan et al., 2017). Suite à cette nouvelle classification, de nombreuses souches du complexe doivent être réassignées, c'est le cas par exemple de la souche *B. amyloliquefaciens* subps. *plantarum* FZB42 devant être renommée *B. velezensis* FZB42 ainsi que la souche *B. subtilis* GB03 devenant *B. velezensis* GB03. Une récente initiative a été mise en place afin de procéder à une réassignation systématique pour les souches dont les séquences sont présentes dans la base de données GenBank (Federhen et al., 2016). La séquence de la souche *B. subtilis* QST713 n'étant pas disponible au démarrage de ma thèse, une incertitude persistait sur sa projection dans la nouvelle classification de ce complexe.

Table 1: Nouvelle classification du « *Bacillus subtilis* species complex » (Fan et al., 2017).

Clades	**Espèces**
I: « *subtilis* »	*B. subtilis* subps. *subtilis* *B. subtilis* subsp. *spizenii* *B. subtilis* subsp. *inaquosorum* *B. tequilensis* *B. vallismortis* *B. mojavensis* *B. atrophaeus*
II: « operationnal group *B. amyloliquefaciens* »	*B. amyloliquefaciens* subsp. *amyliloquefaciens* *B. siamensis* *B. velezensis consortium* (*B. velezensis, B. amyloliquefaciens* subsp. *plantarum, B. methylotrophicus*)
III: « *licheniformis* »	*B. licheniformis* *B. sonorensis*
IV: « *pumilus* »	*B. pumilus* *B. safensis* *B. xiamenensis* *B. altitudinis* *B. stratosphericus* *B. aerophilus*

L'utilisation d'espèces *B. subtilis/amyloliquefaciens* comme agents de biocontrôle est le plus souvent associée à leur capacité d'inhibition de flores pathogènes de cultures, mais également à la possibilité de développer des produits commerciaux stables formulés à partir de leurs spores (Branda et al., 2004; Borriss, 2015).

La souche *B. subtilis* QST713 est le principe actif de nombreux produits de biocontrôle mis sur le marché mondial par la société AgraQuest rachetée en 2012 par le géant allemand Bayer CropScience (EPA Office of Pesticide Programs, 2000). Cette souche est utilisée comme agent de biocontrôle dans de nombreuses cultures dans le but de traiter les maladies de plantes liées principalement aux pathogènes fongiques ainsi qu'aux pathogènes bactériens (EPA Office of Pesticide Programs, 2000). La souche QST713 est inscrite en tant que substance active à l'annexe I de la directive 91/414/CEE par la directive 2007/6/CE traitant de la mise sur le marché de produits phytopharmaceutiques (La commission des Communautés européennes, 2007).

Les mécanismes de bioprotection de cette souches stipulés dans le document d'action pour l'enregistrement du biopesticide (BRAD : Biopesticide Registration Action Document) seraient (i) la compétition nutritionnelle, (ii) l'exclusion de niche, (iii) la colonisation, (iii) l'attachement des bactéries aux pathogènes fongiques et (iv) l'induction des défenses naturelles des plantes (EPA Office of Pesticide Programs, 2000). D'après ce rapport technique, la souche QST713 pourrait empêcher la germination de spores de pathogènes, perturber la croissance des tubes germinatifs et inhiber l'attachement des pathogènes aux plantes (EPA Office of Pesticide Programs, 2000). Cependant, son génome est encore inconnu des banques de données et peu de publications scientifiques se sont focalisées sur les mécanismes associés à ses capacités de bioprotection (Joshi and McSpadden Gardener, 2006; Lahlali et al., 2011, 2013; Patel et al., 2011; Cawoy et al., 2015; Punja et al., 2016).

L'antagonisme dû à la production de métabolites secondaires par les agents de biocontrôle est un des principaux mécanismes étudiés dans le domaine de biocontrôle agricole (Ongena et al., 2005; Ongena and Jacques, 2008; Cawoy et al., 2015). Les *Bacillus* produisent de nombreux métabolites secondaires ayant une activité antimicrobienne, 4 à 5% du génome de la bactérie *B. subtilis* est alloué à la production de ces composés et 8,5% du génome de *B. velezensis* FZB42 (Stein, 2005; Chen et al., 2009; Zhao and Kuipers, 2016). L'étude de Zhao & Kuipers (2016) décrit les principaux métabolites secondaires pouvant avoir une activité antimicrobienne chez les Bacillales ; les principaux types étant les peptide synthétisés par voie ribosomique avec modifications post-traductionnelles (RiPP : Ribosomally synthesized and post-translationally modified peptides), les polycétides synthétisés par les polycétides synthétases (PKS : Polyketides synthetases), les peptides non-ribosomiques synthétisés par les synthétases de peptides non-ribosomiques (NRPS : Non-Ribosomal Peptide Synthetases), et les hybrides PKS-NRPS (Raaijmakers et al., 2010; Wang et al., 2014; Zhao et al., 2014; Aleti et al., 2015; Weissman, 2015; Palazzini et al., 2016; Zhao and Kuipers, 2016). *Bacillus subtilis* produit la subtiline, un RiPP de la famille des lantipeptides ayant une activité antibactérienne contre les bactéries à Gram positif par formation de pores dans la membrane cytoplasmique (Breukink et al., 1999; Parisot et al., 2008). De nombreux *Bacillus* (*subtilis*/ *amyloliquefaciens*/*velezensis*...) produisent les lipopetides (LP) NRPS, dont la fengycine (Guo et al., 2014; Luo et al., 2015), la surfactine (Bonmatin et al., 2003; Kim et al., 2010; Pathak and Keharia, 2014; Luo et al., 2015) et la bacillomycine D du groupe des iturines (Tenoux et al., 1991; Moyne et al., 2001), ayant des activités antifongiques (Zhao et al., 2014; Aleti et al., 2015). De nombreux *Bacillus* produisent la bacylisine, un NRPS connu pour avoir une activité antibactérienne (Chen et al., 2009b; Özcengiz and Öğülür, 2015; Palazzini et al., 2016). Entre autres, les *Bacillus amyloliquefaciens/velezensis* produisent les PKS difficidine, macrolactine et bacillaene ayant des activités antibactériennes (Chen et al., 2006, 2009b; Aleti et al., 2015; Chowdhury et al., 2015).

Des études récentes ont posé l'hypothèse que les mécanismes de biocontrôle liés aux molécules antimicrobiennes seraient davantage liés à leurs effets éliciteurs des défenses naturelles des plantes,

plutôt qu'à leurs activités antimicrobiennes (Raaijmakers et al., 2010; Chowdhury et al., 2015a, 2015b). Ces articles illustrent notre manque de connaissance sur le fonctionnement intime de ces procédés de biocontrôle qui sont aujourd'hui encore le plus souvent mis en en œuvre de manière empirique. Clairement, des études mécanistiques sont nécessaires aujourd'hui pour décrypter la complexité des mécanismes biologiques mis en jeu pour mieux les contrôler et accompagner les politiques de déploiement de ces approches agricoles durables.

Chapitre 2: Prise en compte de la formation de biofilm dans les mécanismes de biocontrôle

Préambule

Les microorganismes peuvent vivre selon différents modes de vie. C'est le cas des bactéries qui peuvent alterner un mode de vie libre « planctonique » avec un mode de vie sessile et social sur les surfaces « le biofilm ». Ce dernier est caractérisé par une organisation spatiale de microorganismes englués dans une matrice organique extracellulaire et associés à une surface biotique ou abiotique. La notion de « biofilm » apparait pour la première fois en 1978. Elle est décrite par J. W. Costerton comme étant le mode de vie le plus répandus des microorganismes dans leurs environnements naturels (Costerton et al., 1978, 1981, 1994).

La formation des biofilms sur une surface se déroule en plusieurs étapes plus ou moins séquentielles (Fig. 10) (Vlamakis et al., 2013; Tremblay et al., 2014; Flemming et al., 2016). L'adhésion est la première étape de formation d'un biofilm sur une surface. Elle dépend d'intéractions physico-chimiques entre la paroi des bactéries et le support mais également de la présence de décorations de surface telles que les flagelles, fimbriae, pili. Elles passeront donc d'un état planctonique à un état sessile via une étape d'adhésion initialement réversible évoluant vers une adhésion irréversible. Une fois adhérées, les cellules vont proliférer et former des agrégats appelés microcolonies. Au sein de ces microcolonies, les cellules vont libérer les constituants de la matrice extracellulaire, principalement composée d'eau, de polysaccharides, de lipides et d'ADN (acide désoxyribonucléique) extracellulaires (Flemming et al., 2007; Flemming and Wingender, 2010). Cette matrice va permettre d'interconnecter les cellules et de stabiliser le biofilm par formation d'un réseau de polymères structurés tridimensionnellement lors de la maturation du biofilm. Cette matrice peut apporter protection et nutrition aux cellules en cas de stress extérieurs (Flemming et al., 2016). Le cycle du biofilm est dynamique. Une fois la structure formée, une étape de dispersion du biofilm peut s'enclencher pour permettre une dissémination efficace de la colonie et assurer leur survie. Ce détachement peut être induit soit par les bactéries elles-mêmes, soit par des perturbations environnementales externes, telles que (i) des perturbations mécaniques comme l'abrasion du biofilm, (ii) des perturbations biochimiques comme la dégradation de la matrice ou du substrat par des enzymes, et également (iii) des perturbations biologiques causées par les interactions entre organismes dans l'environnement (Kaplan, 2010).

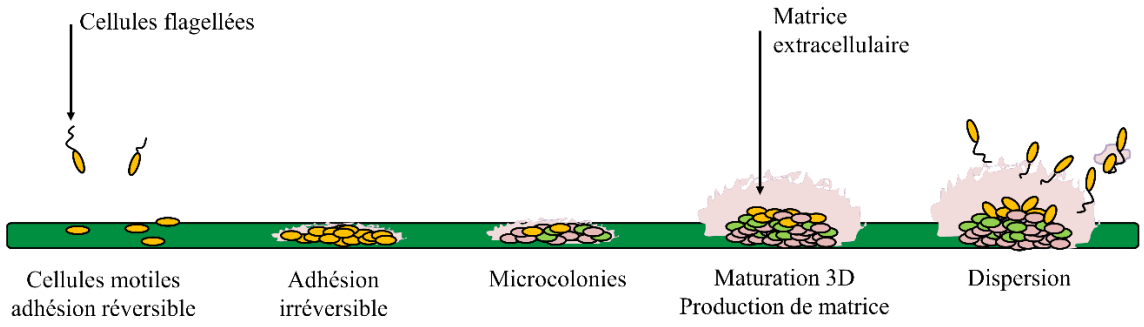

Fig. 10: Schéma simplifié des étapes de formation d'un biofilm.

Actuellement, en agriculture, les systèmes de protection des plantes les plus utilisés sont les pesticides chimiques. Comme nous l'avons vu précédemment, en France, avec le plan EcoPhyto 2, l'utilisation de ces pesticides devra être progressivement remplacée par des solutions moins radicales et

respectueuses de l'environnement comme l'utilisation de microorganismes comme agents de biocontrôle.

A travers les deux articles suivants, nous allons tout d'abord examiner les capacités d'adaptation et de réorganisation spatiale des bactéries sous forme de biofilm face aux fluctuations environnementales *via* l'article de revue, « Spatial organization plasticity as an adaptative driver of surface microbial communities » publié dans *Frontiers in Microbiology* en 2017. Dans un second temps, nous allons explorer l'intérêt et les conséquences du mode de vie en biofilm pour les bactéries bénéfiques dans le cadre de la bioprotection des systèmes agricoles à travers l'article de revue « Should the biofilm mode of life be taken into consideration for microbial biocontrol agents ? » paru dans *Microbial Biotechnology* en 2017.

2.1 Article 1: "Spatial organization plasticity as an adaptive driver of surface microbial communities"

Arnaud Bridier[1], Jean-Christophe Piard[2], Caroline Pandin[2], Simon Labarthe[3], Florence Dubois-Brissonnet[2], Romain Briandet[2]

[1] Fougères laboratory, Anses, France,
[2] Micalis, INRA-AgroParisTech-Université Paris-Saclay, France,
[3] MaIAGE, INRA-Université Paris-Saclay, France

Frontiers in Microbiology, 2017

(http://dx.doi.org/10.3389/fmicb.2017.01364)

frontiers in Microbiology

REVIEW
published: 20 July 2017
doi: 10.3389/fmicb.2017.01364

Spatial Organization Plasticity as an Adaptive Driver of Surface Microbial Communities

*Arnaud Bridier[1], Jean-Christophe Piard[2], Caroline Pandin[2], Simon Labarthe[3], Florence Dubois-Brissonnet[2] and Romain Briandet[2]**

[1] Antibiotics, Biocides, Residues and Resistance Unit, Fougères Laboratory, ANSES, Fougères, France, [2] Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France, [3] MaIAGE, INRA, Université Paris-Saclay, Jouy-en-Josas, France

Biofilms are dynamic habitats which constantly evolve in response to environmental fluctuations and thereby constitute remarkable survival strategies for microorganisms. The modulation of biofilm functional properties is largely governed by the active remodeling of their three-dimensional structure and involves an arsenal of microbial self-produced components and interconnected mechanisms. The production of matrix components, the spatial reorganization of ecological interactions, the generation of physiological heterogeneity, the regulation of motility, the production of actives enzymes are for instance some of the processes enabling such spatial organization plasticity. In this contribution, we discussed the foundations of architectural plasticity as an adaptive driver of biofilms through the review of the different microbial strategies involved. Moreover, the possibility to harness such characteristics to sculpt biofilm structure as an attractive approach to control their functional properties, whether beneficial or deleterious, is also discussed.

Keywords: microbial biofilm, spatial dynamic, structure/function, adaptative response

OPEN ACCESS

Edited by:
Sara María Soto, ISGlobal, Spain

Reviewed by:
Akos T. Kovacs, Technical University of Denmark, Denmark
Giovanna Batoni, University of Pisa, Italy

****Correspondence:***
Romain Briandet
romain.briandet@inra.fr

Specialty section:
This article was submitted to Infectious Diseases, a section of the journal Frontiers in Microbiology

Received: *30 March 2017*
Accepted: *05 July 2017*
Published: *20 July 2017*

Citation:
Bridier A, Piard J-C, Pandin C, Labarthe S, Dubois-Brissonnet F and Briandet R (2017) Spatial Organization Plasticity as an Adaptive Driver of Surface Microbial Communities. Front. Microbiol. 8:1364. doi: 10.3389/fmicb.2017.01364

INTRODUCTION

The traditional perception of microbes as unicellular life forms has deeply changed over the last decades with the collection of scientific evidences showing that microorganisms predominantly live in dense and complex communities known as biofilms. Biofilms are classically defined as aggregates of cells adhering to a surface or interface and often embedded in an extracellular matrix of polymeric substances. They constitute one of the most successful mode of life on Earth (Flemming et al., 2016). They are consequently found in natural, industrial, medical, household environments and, from the human point of view, they can be either beneficial or detrimental. Indeed, microbial biofilms are involved in essential nutrient cycling or biotechnological processes as well as in severe chronic infections and biodeterioration phenomenon (for instance Beech and Sunner, 2004; Bjarnsholt, 2013; Berlanga and Guerrero, 2016). Positive or negative impacts directly result from the ability of microorganisms to express specific functions in these complex communities compared to the single planktonic state. The higher resistance of biofilm cells to antimicrobials compared to that of their planktonic counterparts is a telling example of such specific functional properties and should be relied to the structural characteristics of the community (Bridier et al., 2011). Indeed, both the microbial growth and the production of matrix lead to the rise of a biological edifice offering progressively a protective structure to inhabitants

able to hinder penetration and action of antimicrobials. The development of three-dimensional biofilm structure also generates physicochemical gradients and physiological heterogeneity with slow growth resistant phenotypes for instance (Stewart and Franklin, 2008). Recently, Berleman et al. (2016) demonstrated the central role of multicellular bacterial community structure in the colonization of surface by *Myxococcus xanthus*. Indeed, the authors showed that extracellular polymeric substances (EPS) synthesis led to the creation of microchannels which govern both bacterial motility and cell-to-cell interactions and finally organize multicellular behavior during swarm migration. In contrast, a mutant lacking EPS showed a deficiency of cell orientation and poor colony migration. As biofilms are mostly complex associations of strains and/or species in our environments, spatial arrangement of genotypes within biofilms also governs strain interactions and the evolution of social phenotypes as immediate neighbors in the structure are more affected by the social behaviors (Nadell et al., 2016). Spatial organization of genotypes and social interactions will thus govern the whole community architecture and functions (Liu et al., 2016). Functional properties of a biofilm therefore emerge from the construction and shaping of the microbial structure like many of the emergent properties of natural communities relying on the creation of biogenic structures by habitat-forming organisms (Flemming et al., 2016).

The close relationships between the architecture of a biofilm and its functional properties emphasizes the need to better describe and understand cell behavior, from single cell to multicellular scale, during biofilm structure development and maturation. Recent technological advances in methodologies including imaging and microscopy, molecular techniques, and physico-chemical assays, enabled the development of novel approaches dedicated to biofilm studies (Azeredo et al., 2017). The possibility to observe biofilm using high resolution and non-destructive methods now allows investigating the dynamics of multicellular structure development and the fate of each of its individual cellular components in parallel. For instance, the key architectural transitions and associated biophysical and genetic mechanisms supporting the developmental program of *Vibrio cholerae* biofilms have been recently disclosed using single-cell live imaging (Drescher et al., 2016; Yan et al., 2016). This kind of observations has clearly improved our understanding of spatio-temporal development of biofilms and has finally increasingly supported the intimate connection between structural modulations and the emergence of functional features and survival strategies. Indeed, the ability of biofilms to adapt their structure in response to internal or external stimuli, called hereafter the architectural plasticity, appears as a key factor affecting the fitness of individuals within the whole microbial community. Interestingly, the role of plasticity in bacterial survival was already demonstrated at the cellular scale. Bacteria are able to alter their morphology and to produce specific morphotypes conferring survival advantages in hostile environments. This was showed for numbers of bacterial pathogens for which filamentation is essential in the resistance to phagocytosis and overall for persistence during infection (Justice et al., 2008; Justice et al., 2014).

In this review, we will discuss the central role of architectural plasticity in the emergence of functional properties of biofilms and as a communal bacterial response to many harsh conditions and external attacks. We will also deal with the various mechanisms developed by microorganisms to build and modify the three-dimensional community and, with the existing strategies for humans to sculpt biofilm architecture in order to control their function.

BIOFILM ARCHITECTURE PLASTICITY AS A COLLECTIVE RESPONSE TO ENVIRONMENTAL FLUCTUATIONS

The starting point of the development of the three-dimensional biofilm structure corresponds to the transition from planktonic state to sessile mode of life which occurs in response to diverse environmental cues and cell-to-cell signaling molecules. The translation of perceived signals to specific genetic expression and finally to a series of dramatic metabolic and phenotypic changes involves complex regulatory networks and diverse molecules including the second messenger cyclic-di-GMP (c-di-GMP) in number of bacterial species (Kostakioti et al., 2013; Romling et al., 2013). A correlation between high intracellular levels of c-di-GMP and biofilm formation has indeed been shown for a variety of species and various biofilm determinants including flagella rotation, exopolysaccharide production, surface adhesin expression, secondary metabolite production, antimicrobial resistance and other stress responses (Valentini and Filloux, 2016). In addition, the quorum sensing (QS), which is a cell-to-cell signaling system making bacteria able to communicate with each other via the production and detection of signaling molecules, enable the regulation of communal behaviors (Srivastava and Waters, 2012). The interconnection between QS and c-di-GMP pathways enables bacteria to act collectively through coordinated response to cellular signals or environmental conditions. It was showed for instance in *V. Cholerae* that QS and c-di-GMP pathways are strongly intertwined at many levels and that their integration play a key role in the control of the expression of *vpsT*, a transcriptional activator that induces biofilms formation (Srivastava et al., 2011). Similarly, Ueda and Wood (2009) demonstrated in *Pseudomonas aeruginosa* that the transcription of the *tpbA* gene encoding a tyrosine phosphatase involved in synthesis of polysaccharides and biofilm formation, is under the direct control of QS while this enzyme is also involved in the regulation of intracellular c-di-GMP concentrations. Such observation clearly highlights the convergence of the two signaling processes and the connection between the environment, cell populations and finally biofilm formation.

Using this sensor system, bacteria are able to coordinate their activities during the different steps of biofilm development leading to complex three-dimensional structures. Recurrent developmental stages can be schematically defined in bacteria ranging from initial adhesion to irreversible attachment, formation of microcolonies, macrocolonies development and

maturation of architecture and then dispersion (Monds and O'Toole, 2009). Nevertheless, the development program and its dynamic are actually very specific and largely depend on nutrient conditions, pH, temperature, hydrodynamics conditions, species involved, etc. . . Fundamentally, the shaping of specific biofilm architecture reflects the impact of local growth conditions (Toyofuku et al., 2015). Numerous studies in various bacterial species reported the impact of temperature, hydrodynamics or nutrient concentration on biofilm structure suggesting an adaptation of biofilm shaping to optimally fit to growth conditions (Stoodley et al., 1998; Yang et al., 2006; Abdallah et al., 2015). The changes of biofilm structure alter the diffusibility of substances and enables metabolic adaptation under various conditions by optimizing nutrient and waste product exchange for instance (Toyofuku et al., 2015).

This is illustrated in **Figure 1** where confocal images of biofilms with various architectures were used as an input of a modeling pipeline, which simulates diffusion of a chemical molecule through biofilm and thus reflects its diffusive capabilities. The diffusion coefficient maps obtained suggested that biofilm architecture is a determinant driver of the chemical compound density map at steady state, presenting a diversity of situations, from quasi-uniform distributions to strong gradients.

Accordingly, it is clear that structural adjustments of biofilm clearly lead to both the modulation of phenotypic heterogeneity and the way each bacterium perceive its local microenvironment. This architectural plasticity provides thereby an efficient way to adapt to various stresses for microorganisms. Many demonstrations of this phenomenon occur in our environments as for instance, stream biofilms in rivers, which dynamically adapt and evolve in response to the streambed environment and flow intermittency through modifications of their physical structure, species composition and through spatial re-organization (Battin et al., 2016; Sabater et al., 2016). The intimate relation between architectural differentiation and community composition suggests that this micro-scale process is an important driver of the biofilm adaptation to the fluctuations of stream conditions, especially to compensate hydrodynamic perturbations and changes in quantity and quality of nutrients (Besemer et al., 2009).

Another concrete illustration of adaptation through biofilm structure modulation is the stimulation of biofilm production in different bacterial species exposed to antimicrobials, metals and a large range of other molecules (Hoffman et al., 2005; Perrin et al., 2009; Shemesh et al., 2010; Marchal et al., 2011; Chen et al., 2015). In many cases, the presence of subinhibitory concentration of such toxic molecules induces the sur-expression of genes coding for matrix components that finally lead to an increase of biofilm production and a modification of its three-dimensional structure (Shemesh et al., 2010). In line with this, it was showed in *Thiomonas* sp. that arsenic exposure lead to an increase of EPS production and cell death within microcolonies creating hollow voids structure that is subsequently followed by active dispersal of cells (Marchal et al., 2011). Authors suggested that the survival and persistence of *Thiomonas* sp. under selective pressure of arsenic exposure relied on its ability to rapidly develop biofilm followed by the dispersal of a more resistant population.

Architectural plasticity of biofilms thus gives the opportunity to bacteria to constantly reorganize their direct microenvironments to face adverse conditions and to better harness surrounding resources (**Table 1**). Structural adaptations can occur through various active processes which mostly involve a differential expression of genes or a genetic plasticity in response to conditions changes. The diverse mechanisms, directly or indirectly involved in the shaping of biofilm architecture, are discussed in the next section.

MICROBIAL SYSTEMS TO SHAPE BIOFILM STRUCTURE

Microorganisms harness an arsenal of complementary mechanisms to tailor biofilm architecture. They range from regulation of cell motility to modification of cellular morphology, production of matrix components, generation of genetic and physiological heterogeneity or subpopulation interactions. Examples of modulations of biofilm architecture in response of various environmental conditions and depending on bacterial composition are displayed in **Figure 2**.

Genetic and Physiological Adaptation at Single Cell Scale

Physical and chemical microenvironments within the biofilm (e.g., varied conditions of pH, osmotic strength, nutrients or exposure to sublethal concentrations of biocide) induce heterogeneous metabolic activity and adaptive responses among biofilm cells (Bridier et al., 2011; Giaouris et al., 2015). During biofilm development, the population displays multiple phenotypes (Sauer et al., 2002). At the single cell scale, the diversity of cell properties are due to either the phenotypic adaptation driven by up- or down- regulation of gene expression, or the appearance of genetic mutants driven by an increased level of mutation in biofilm environment.

Gene regulation at different stages of biofilm formation, compared to the free-living mode of life, can be studied through the comparison of transcriptomic (Waite et al., 2006; Moreno-Paz et al., 2010; Guilhen et al., 2016), proteomic (Sauer, 2003; Resch et al., 2006; Vilain and Brozel, 2006; Giaouris et al., 2013; Qayyum et al., 2016) or metabolomic (Wong et al., 2015; Stipetic et al., 2016) profiles revealing up- or down- regulated functions. For example, in mature biofilms of *P. aeruginosa*, more than 50% of proteins are upregulated and more than 100 proteins are *de novo* synthesized in comparison to planktonic cells (Sauer et al., 2002). The multiple phenotypes described in biofilm communities do not correspond to a simple mixture of planktonic cells at different growth stages. The biofilm proteome of *Bacillus cereus* was for example demonstrated as unique and different from those of exponential and stationary-phase planktonic cells (Vilain and Brozel, 2006). Compared lipidomics between planktonic and biofilm cells also support the idea of specific biofilm phenotypes. Indeed, in various growth conditions, the biofilm cell membrane of different bacterial

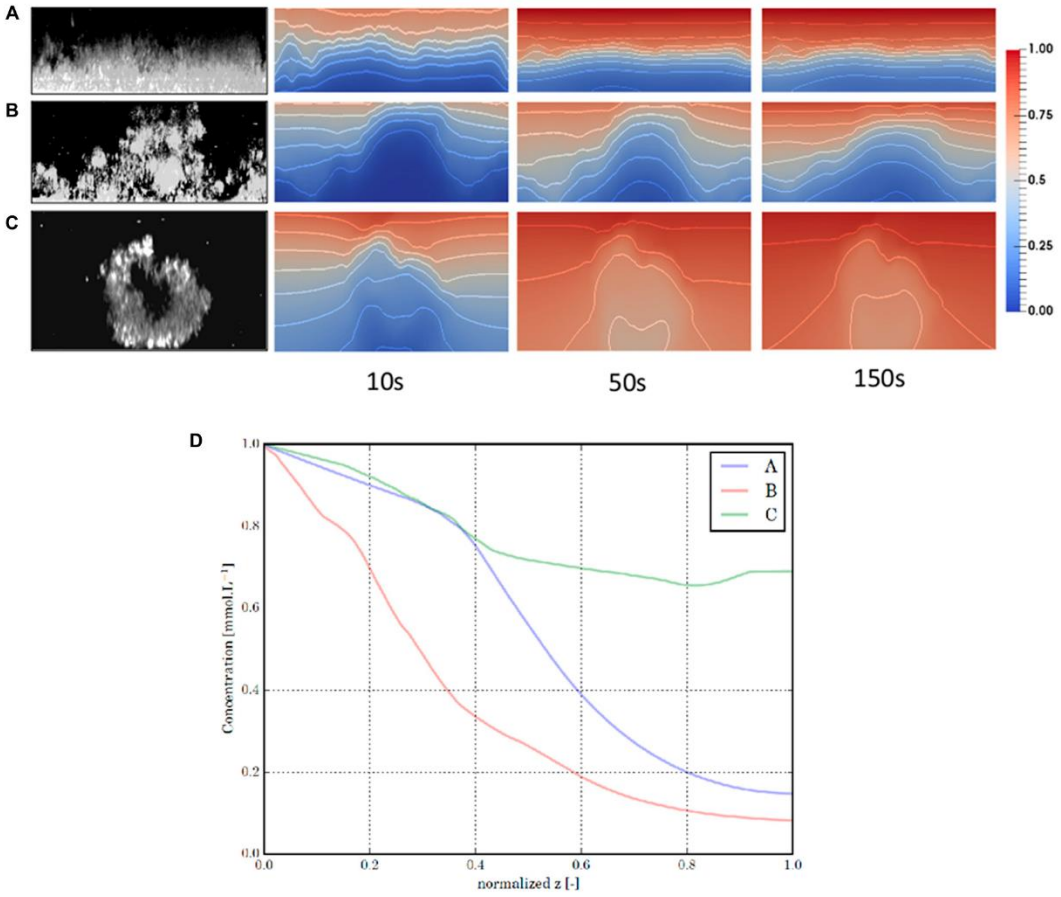

FIGURE 1 | Modeling of diffusion in biofilm of various architecture. CLSM sections of three characteristic biofilm structures were displayed in the first column: **(A)** a flat (*Escherichia coli*), **(B)** a mushroom-like (*Pseudomonas putida and Pseudomonas aeruginosa*) and **(C)** a egg-like structure (*Salmonella enterica*). Those images are used as an input of a modeling pipeline which simulates the diffusion of a chemical component through the biofilm, from a bulk source located in the upper boundary of the image. Based on the biofilm images, we construct for each structure a heterogeneous diffusion coefficient map that reflects the diffusive capabilities of the biofilm: the higher the local bacterial density, the lower the local diffusion coefficient. Next, this tensor is inserted in a reaction-diffusion equation together with a reaction function that mimics the consumption of the component by the bacteria. The consumption rate also varies with the local bacterial density. We display three snapshots of the simulated component distribution, at time t = 10, 50, and 150 s when the steady-state is reached. Isolines are displayed every 0.1 to better represent the distribution gradients. We finally display a cut in z of the component distribution in each biofilm at steady-state **(D)**. The cut plane of a given biofilm crosses its point of minimal component concentration at steady-state. To facilitate the comparison, we normalized the z-coordinates of the different graphs. We can see that the biofilm structure is a determinant driver of the component density map at steady-state, presenting a diversity of situation, from quasi-uniform distributions (structure C) to strong gradients (structures A and B).

strains was shown to be more saturated than their planktonic counterparts, whatever their growth phase (Dubois-Brissonnet et al., 2016). In addition, the spatialized environments in biofilms promote the generation and fixation of a phenotypic diversity compared to selection of only one or very few clones in well mixed environment (Traverse et al., 2013; Martin et al., 2016). The spatial distribution of the biofilm multiple phenotypes can be visualized within the biofilm thickness through the observation of different patterns of physiological characteristics (growth rate, mRNA, proteins synthesis or CsgD production) using for example Gfp reporter systems (Werner et al., 2004; Lenz et al., 2008; Stewart and Franklin, 2008; Serra et al., 2013).

Regulation of genes which differentiate planktonic and biofilm protein patterns are numerous and can be partitioned in several categories: metabolism (carbon catabolism, aerobic/anaerobic metabolism, membrane and transport), stress responses and adaptation, motility and attachment (flagellin, surface proteins), EPS production and quorum-sensing signaling (Whiteley et al., 2001; Sauer et al., 2002; Khemiri et al., 2016). Both transcriptional and post-transcriptional regulation occur: the first is slow but may be important for the long-term stability of the biofilm

TABLE 1 | Examples of biofilm structural responses to environmental fluctuations associated with the alteration of community functions.

Biofilm composition	Environmental fluctuation	Structure alteration	Impact on functional properties	Reference
Bacillus subtilis 3610	Exposition to sublethal dose of chlorine dioxide (ClO_2)	Increased matrix production and acceleration of biofilm formation	Partial protection against ClO_2	Shemesh et al., 2010
Bacillus subtilis 3610	Exposition to bacilli relatives isolated from soil	Increase in matrix-producing cannibals subpopulation, matrix induction	Hypothetical increase survival within a multispecies biofilm	Shank et al., 2011
Thiomonas sp. CB2	Exposition to subinhibitory dose of arsenite	Increased production of extracellular polysaccharides and creation of hollow voids containing motile cells	Increased protection to As(III)	Marchal et al., 2011
Pseudomonas fluorescens PCL1701	Exposition to calcium ions ($CaCl_2$)	Increase biofilm surface coverage, biovolume	Reduced stiffness, higher viscous effect, larger adhesive values at the surface of the biofilm	Safari et al., 2014
Stream biofilms	Exposition to flow intermittency	Changes of physical structure, community composition and spatial arrangement	Adaptation of ecosystem metabolism	Battin et al., 2016; Sabater et al., 2016
Gravity sewer biofilms	Increasing shear stress	Increase porosity of the biofilm	Reduction in the chemical oxygen demand	Xu et al., 2017
Xanthomonas axonopodis (citrus bacterial canker)	Exposition to *Bacillus subtilis* or *Bacillus* TKS1-1 *amyloliquefaciens WG6-14*	Alteration of the spatial repartition and density of the pathogen in the multispecies biofilm	Citrus leaves protection from the plant pathogen	Huang et al., 2012
Burkholderia cenocepacia	Exposition to the free-living ciliate *Tetrahymena pyriformis*	Increase of biofilm production and formation of specific round-shape microcolonies	Resistance to protozoan grazing	Kaminskaya et al., 2007
Fouling biofilm developed on ultrafiltration membrane	Exposition to the protozoa *Tetrahymena pyriformis*	Shift in biofilm structure from flat to aerial and porous 3D organization	Permeate fluxes in the presence of the predators increased by 2	Derlon et al., 2012
Fouling biofilm developed on filtration membrane	Exposition to metazoan worms (nematodes or oligochaetes)	Shift in biofilm structure from flat to aerial and porous 3D organization	Increase of permeate fluxes in the presence of the predators	Klein et al., 2016
Staphylococcus aureus RN4220	Exposition to bacilli swimmers	Vascularisation of the biofilm matrix	Sensitization to biocide action	Houry et al., 2012
Streptococcus pyogenes SP5	Exposition to fluoroquinolone derivatives	Modulation of EPS production and biofilm architecture	Sensitization to the antibiotic treatment	Shafreen et al., 2011
Staphylococcus epidermidis	Exposition to Dispersin B (beta-*N*-acetylglucosaminidase)	Hydrolyze of the glycosidic linkage of the extrapolysaccharidic matrix, biofilm dispersion	Potentialisation of antibiotic (cefamandole nafate) action	Donelli et al., 2007
Water system multispecies biofilm	Exposition to sodium nitroprusside (NO donor)	Drastic reduction in 3D organization	Partial loss of chlorine tolerance	Barraud et al., 2009
Listeria monocytogenes	Exposition to DNase I and proteinase K	Disruption of the biofilm matrix, loss of 3D organization	Decrease of persistence on industrial surfaces	Nguyen and Burrows, 2014
Pseudomonas aeruginosa	Exposition to biosynthetic glycoside hydrolases PelAh and PslGh	Disruption of the biofilm spatial organization	Potentialisation of colistin and neutrophils	Baker et al., 2016
Wound biofilms, *Staphylococcus epidermidis*	Exposition to EDTA (Ethylenediaminetetraacetic acid)	Disruption of biofilm structure	Potentialisation of antimicrobials	Finnegan and Percival, 2015; Maisetta et al., 2016

(Guttenplan and Kearns, 2013); the second is described to be mainly controlled by the c-di-GMP intracellular level. As mentioned previously, elevated intracellular levels of c-di-GMP generally promote EPS synthesis and biofilm formation, while decreased levels reduce biofilm formation (Martinez and Vadyvaloo, 2014).

Besides, biofilms can constitute an optimal environment for both cell to cell exchanges of genetic material and genetic

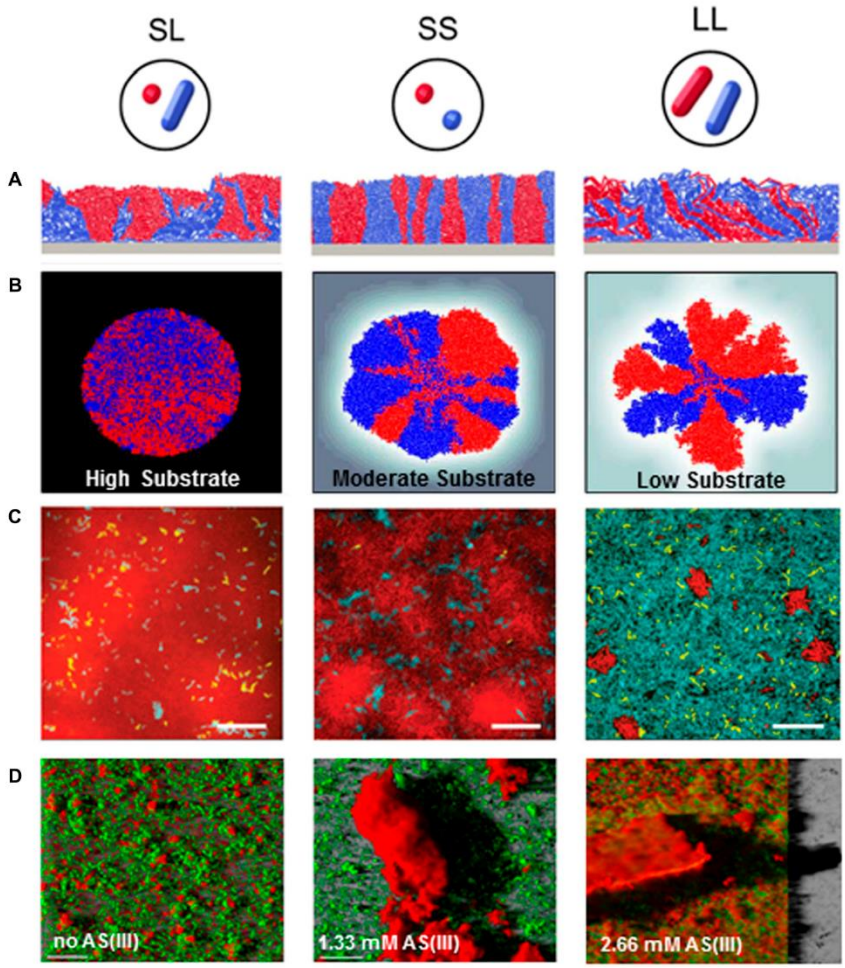

FIGURE 2 | Biofilm architectural modulations in response to environmental stimuli or depending on bacterial composition. **(A)** Impact of cell morphology on biofilm spatial organization. Images displayed 2D sections from simulations where biofilms exponentially grown from 1:1 mixtures of red- and blue-labeled strains form distinct 3D patterns depending on the coccal (S) or rod-like (L) morphology of the strain (Adapted from Smith et al., 2017). **(B)** Impact of substrate availability (High, moderate and low availability) on biofilm architecture and lineage segregation. Simulations were started with a 1:1 mixture of red and blue cells, where cell color served a neutral marker for lineage segregation. Substrate concentration decrease was associated to a higher spatial segregation of cell lineages (Adapted from Nadell et al., 2010). **(C)** Impact of disturbance frequency on *Vibrio cholerae* biofilm spatial organization and strain competition. Images are optical sections taken from the bottom cell layer of biofilms initiated with a 1:1:1 mixture of wild-type strain (teal): a mutant strain hyper-secreting biofilm matrix (red): a mutant strain that is unable to produce extracellular matrix (yellow) cells in microfluidic devices (scale bar: 20 μm). Biofilms grew under continuous nutrient provision (left), or underwent disturbance events every 12 h (middle) or every 6 h (right). Each disturbance event consist in stopping the flow during 2 h to lead to nutrient limitation. Cells were then allowed to disperse to a new microfluidic chamber by pumping the dispersed cells from the initial chamber to the new chamber. After a 2 h-incubation, flow was resumed to pump fresh medium in the newly colonized chamber and enable biofilm growth (adapted from Yan et al., 2017). **(D)** Impact of toxic on EPS production and biofilm structure in *Thiomonas* sp. CB2. Images show three dimensional confocal reconstruction of 7 day-old biofilms cultivated in the absence, or in the presence of 1.33 and 2.67 mM Arsenic [As(III)]. Biofilms were stained using SYTO9 (cells, green) and ConA (exopolysaccharides, red) (Adapted from Marchal et al., 2011).

mutations in biofilm inhabitants. They offer a panel of ideal characteristics for horizontal gene transfer through conjugation and transformation. These include the presence of high cell density favoring physical contact between biofilm bacteria and of a matrix that is rich in communication signals and in extracellular DNA (eDNA) (Madsen et al., 2012). In addition to providing transferable genetic elements (Hannan et al., 2010), the eDNA plays a central role in triggering natural competence in biofilm bacteria (Molin and Tolker-Nielsen, 2003). Horizontal gene transfer has therefore been described in several studies revealing that conjugation levels were 700–1000-fold higher in biofilms compared to planktonic bacterial cells (Król et al.,

2013; Savage et al., 2013). This "permeability" of biofilm bacteria to heterologous mobile genetic elements is likely to shape the evolution of biofilm bacteria and to enhance their relatedness (Madsen et al., 2012). Another mechanism yielding genetic evolution in biofilms is linked to a higher mutation rate of certain biofilm bacteria. Important studies down this line have been performed in *P. aeruginosa*. Initial observations reported that genetic diversification occurred through a *recA*-dependant mechanism within short-term growth in biofilms and yielded mutants with multiple novel traits including motility, nutrition requirements, morphology, biofilm phenotypes, and stress resistance (Boles et al., 2004). The study of mutations *in-situ* within biofilms using a *gfp* gene containing a +1 frameshift mutation showed that mutations occurred in microcolony structures and increased at a frequency 100 to 1800-fold higher than that observed in planktonic cultures (Conibear et al., 2009). The underlying mechanism is linked to the mismatch repair system (MRS) which monitors the fidelity of DNA replication and recombination through its two main components MutS and MutL (Oliver et al., 2002). Using *mut*S deficient derivatives of *P. aeruginosa* and a flow-cell biofilm model system, Luján et al. (2011) showed that the mutants yielded enhanced phenotypic and morphological diversities over wild type strains in structured biofilms. Interestingly, the generated morphotypic variants showed increased competitiveness over the parental strain. This is to correlate to the high prevalence (30–60%) of mutator strains due to alterations in the *mut*S and *mut*L genes in *P. aeruginosa* chronic infections while detection of mutators is rare in *P. aeruginosa* acute infections (Gutiérrez et al., 2004; Feliziani et al., 2010).

Altogether, this overall genetic plasticity of bacteria in biofilm yields a rapid development of diversity among members of biofilm communities and is likely to shape the biofilm structure because of the co-development of the different morphologies and phenotypes. This provides the biofilm with what has been termed the "insurance hypothesis" in ecology that considers that the stability of many biological communities relies on their diversity which increases the chance that some members will be able to withstand environmental variations that the community may encounter (Boles et al., 2004). This enhanced clonal diversity in biofilms is a real challenge in the control of pathogen and detrimental biofilms as they may rapidly adapt to environmental stresses such as treatments with antimicrobials (Macià et al., 2011; Koch et al., 2014; Van Meervenne et al., 2014). In contrast, this diversity is a real benefit in biotechnological issues in which biofilms can be exploited in numerous applications and under many different environmental conditions (Berlanga and Guerrero, 2016; Piard and Briandet, 2016).

Cell Adaptation with Direct Impact on Biofilm Structure

Individual adaptative responses of biofilm cells, due to heterogeneous environments within their complex living place, lead to individual phenotypic changes, such as individual cell morphology and motility or modification of matrix production.

Bacterial motility, within or outside the biofilm structure, is a major driver of the community plasticity. Once associated to a surface, most of the bacterial cells transfer from a motile to a non-motile state. *P. aeruginosa* for example becomes non-motile as soon as it attaches irreversibly to a surface and forms clusters with non-motile cells during the maturation of the biofilm (Sauer et al., 2002). In accordance, transcriptional profiles of *P. aeruginosa* biofilms showed that motility genes are downregulated compared to planktonic cells (Whiteley et al., 2001). The *B. subtilis* motility is also inhibited under biofilm conditions (Guttenplan et al., 2010). In the short-term, motility is inhibited at multiple levels through accumulation of intracellular c-di-GMP (Ahmad et al., 2013; Guttenplan and Kearns, 2013). In the longer term, regulation relies on transcriptional repression which is slow but may be important for the long-term stability of the biofilm (Guttenplan and Kearns, 2013). In mature biofilms, maintenance of motility for the majority of the cells can destabilize multicellular aggregates and regulation of biofilm plasticity likely shifts to other determinants including EPS production (Guttenplan and Kearns, 2013). Nevertheless, some motile minor isogenic subpopulations can coexist with sessile biofilm cells, creating transients pores within a mature biofilm structure, altering the diffusion-limitation properties of the matrix (Houry et al., 2012; Turonova et al., 2015). In *Campylobacter* biofilms, an unusual continued expression of the motility complex was described by proteomics in the whole population which suggests a crucial role of the measured motility in this biofilm phenotype (Kalmokoff et al., 2006). Similarly, flagellar hook protein (FlgE) was expressed in biofilm cells but not in planktonic cells of *Cronobacter sakazakii* (Ye et al., 2016).

Flagella synthesis and movement are highly regulated in response to environmental conditions. During biofilm maturation, starvation stress occurs in the growing biofilm structure, along with a lack of oxygen and accumulation of by-products and QS signaling molecules. All these factors are important drivers of microbial dispersion (Huynh et al., 2012; Martinez and Vadyvaloo, 2014; Solano et al., 2014). Non-coding small RNAs were also recently identified as players of this dissemination process (Chambers and Sauer, 2013). In the well described *P. aeruginosa* biofilm cycle, dispersion is a consequence of the return to a motile state of a subpopulation of bacterial cells in the center of a cluster. This return is possible through phage-mediated localized cell death (hollow-voids) along with the synthesis of enzymes that can degrade extracellular substances (Webb et al., 2003; Sauer et al., 2004). Dispersion is heterogeneously distributed at the surface of the biofilm and can induce modification of the whole biofilm topography. Motility up- and down- regulation is thus an important driver of the biofilm structure plasticity through its role in attachment, cluster formation and disruption.

Besides, individual cell morphology can also have a great impact on the organization of the population within the biofilm consortium (Smith et al., 2017). Growing in biofilm state, some coccoid-shaped bacteria or small rod can elongate and multiple morphotypes of isogenic cells can appear in different layers. Two different shapes of *Lactococcus lactis* were observed in 16 h flow-cell biofilms: coccoid cells were localized in the depth of the structure while a stratum of elongated filaments rises on the interfacial layers of the structure (Perez-Nunez et al., 2011).

Similarly, different morphotypes of uropathogenic *Escherichia coli* were observed from coccoid form to elongated rods, through different stages of biofilm formation. Filamentous bacteria were observed on the edge of late biofilm in connection with detaching cells (Justice et al., 2004). The filamentation was shown to be a response to stressful environment and is essential for uropathogenic *E. coli* virulence (Justice et al., 2006, 2008).

In addition, EPS are the cement of biofilm architecture and their modulation trigger direct alteration of the spatial structure (Ziemba et al., 2016). EPS content includes water and biopolymers originating from biofilm microorganisms including polysaccharides, proteins, lipids, and eDNA (Flemming, 2011; Fong and Yildiz, 2015; Limoli et al., 2015). From an anthropomorphic biofilm perspective, the matrix has been described as the house of bacteria and as such its structure and composition are unique according to the inhabiting bacteria and the environment (Stoodley et al., 1999; Watnick and Kolter, 2000; Flemming et al., 2007; Flemming, 2011). The EPS matrix cannot be considered as a homogeneous slimy material, but rather as the sum of multiple microhabitats with different local environments (oxygen concentrations, pH-values, redox potential, shear forces, etc.). This stratification governs biofilm heterogeneity in which bacterial groups distribute themselves according to their preferred particular microenvironment and to symbiotic relationships (Watnick and Kolter, 2000; Stewart and Franklin, 2008; Flemming, 2011). This heterogeneity in space is doubled by heterogeneity in time: EPS evolves with the biofilm aging and appears as a dynamic structure due to various events including degradation of matrix elements by bacterial enzymes, dissolution of EPS components, incorporation of new material, etc... (Sutherland, 2001). It can also be noted that most of the different components of the matrix are associated by non-convalent interactions suggesting that dissociation can occur through local modifications of the EPS physicochemical properties (pH, ionic strength, hydration, etc...) (Neu and Lawrence, 2016). This poorly characterized plasticity of the EPS matrix makes it the least understood component of biofilms biology and as such has been termed the "dark matter" of biofilms (Flemming and Wingender, 2010; Flemming et al., 2016).

In an attempts to characterize the signals governing matrix formation and modification in biofilms, Shemesh et al. (2010) showed that exposure of *B. subtilis* and *P. aeruginosa* to sublethal doses of a biocide (chlorine dioxide, ClO_2) stimulate biofilm formation. The transcription of two major operons involved in matrix production [*epsA-epsO* involved in polysaccharide (PS) production and *yqxM-sipW-tasA* involved in amyloid production] was shown to be increased by ClO_2 via the membrane-bound kinase KinC. Interestingly, *kinC* mutants unable to make a matrix were hypersensitive to ClO_2. Another kinase within the *epsA-epsO* operon, the EpsAB tyrosine kinase, is involved in regulation of PS production by a seemingly QS mechanism (Elsholz et al., 2014). The membrane sensor EpsA is able to sense the presence of PS and control kinase activity. In the absence of PS, the kinase is inactivated by autophosphorylation while the presence of PS inhibits autophosphorylation and stimulates the phosphorylation of glycosyltransferases and thereby the synthesis of PS. This positive feedback loop therefore ties PS synthesis to the external concentration of PS. This opens exciting perspectives in applications in which exogenous polysaccharides could be used either as inducers of the biofilm way of life or as modulators of the matrix structure. Also this raises the question whether PS produced by one biofilm bacteria could trigger PS production in another biofilm bacterium. A part of the answer probably relies on the yet unknown specificity of the sensor EpsA toward the different PS produced by a biofilm bacterial community. In an attempt to explore such interbacterial interactions, Shank et al. (2011) investigated whether soil bacteria were able to affect the biofilm development in *B. subtilis*. Using a fluorescent reporter fused to the *tapA* promoter, the coculture screening test showed that most strains able to induce matrix production in *B. subtilis* belonged to the *Bacillus* genus suggesting that interactions occur mostly with close relatives. Two mechanisms were dissected. One involves the activation of the sensor kinase KinD while the other is kinase independent and involves the master regulator Spo0A (Shank et al., 2011).

Species belonging to *Thiomonas* species are frequent in arsenic polluted sites and play key roles in arsenic natural remediation (Marchal et al., 2011). Exposure of *Thiomonas* sp. to sublethal arsenite concentration yielded biofilms with an up to six-fold increase in PS production concomitantly to a 83-fold increase in cell death and cell lysis. This was accompanied with a complex rearrangement of the biofilm structure into PS covered mushroom-like structures in which eDNA was a key player as treatment with a nuclease abolished such phenomenon. eDNA is indeed a crucial component of the biofilm matrix and is involved in multiple interactions with other EPS components including PS and amyloids (Hu et al., 2012; Liao et al., 2014; Schwartz et al., 2016). In *Staphylococcus aureus*, the *cidA* and *lrgA* genes act as holins and antiholins, respectively, and regulate cell lysis in an analogous way to that observed in bacteriophage-mediated cell lysis. While wild-type *S. aureus* produced biofilm with distinct mushroom-like 3D structures that are characteristic of mature biofilms, both a *cidA* mutant deficient in lysis and a *lrg* mutant deficient in the inhibition of CidA-mediated lysis produced biofilms lacking 3D mushroom-like structures (Mann et al., 2009). *S. aureus* is also able to produce and secrete Nuc, a thermostable nuclease. Analysis of the biofilm formed by a *nuc* mutant showed increased amounts of mushroom-like structures. Also, treatment of the *S. aureus* biofilms with DNAseI in flow cell chambers completely removed biofilms. Altogether this suggests that different bacterial factors are able to modulate the level of available eDNA that appear critical in the shaping of biofilm structure and dispersal.

Cell Adaptation with Indirect Changes on Biofilm Structure via Increased Resistance and Persistence

Physiological adaptation of individual cells within the biofilm community may lead to an increased resistance to biocides and antibiotics. Stresses such as starvation (oxygen or nutrients) in the depth of the biofilm or contact with sublethal concentrations of antimicrobials during disinfection can induce a bacterial

stress response and higher tolerance to biocides (Mangalappalli-Illathu et al., 2008; Bridier et al., 2011). The higher individual cell resistance can be explained by several mechanisms. An overexpression of enzymes that are able to degrade biocides (catalase, superoxide dismutase) was described in biofilm under the control of QS (Hassett et al., 1999). *P. aeruginosa* membrane efflux pumps were shown to be up-regulated for cell cultivated in biofilm although their exact role in the biology of these sessile communities needs to be clarified (Zhang and Mah, 2008; Soto, 2013). Moreover, by limiting biocide intracellular penetration, the observed increase in membrane saturation in biofilm cells compared with their planktonic counterparts can be another resistance mechanism (Dubois-Brissonnet et al., 2016). After repeated antimicrobial treatments, the development of the most resistant surviving cells in the biofilm structure will modify the spatio-temporal dimension of the biofilm architecture.

Interactions between Biofilm Subpopulations

Multispecies biofilm is a result of cell–cell and cell–environment interactions such as cooperation, competition or exploitation that create heterogeneity in biofilms (Liu et al., 2016). These specific interactions between species are involved in the spatial organization of biofilms in which they are more favored than in planktonic environments. They maintain their diversity and stability by generating more physiological and functional heterogeneity (Nadell et al., 2009; Pamp et al., 2009; Rendueles and Ghigo, 2015; Kragh et al., 2016; Liu et al., 2016; Pande et al., 2016). Indeed, in specific environment, some species cannot form biofilm alone, but grow in association with others species in multispecies biofilm showing interspecific cooperation interactions between subpopulations (Palmer et al., 2001). Specific interactions and spatial organization within biofilm create fitness effect through social phenotypes. A telling example is the symbiotic two-species consortium formed by *Pseudomonas putida* and *Acinetobacter* sp. strain C6 which has evolved in a non-random spatial organization where *P. putida* exclusively attached and grew on pre-existent colonies formed by *Acinetobacter* sp. strain C6. Resulting evolved communities were characterized by an increased fitness and productivity (Hansen et al., 2007). Microscopic time-lapse observations revealed that cell clusters were arranged according to a uniform pattern and that such structure results from the moving along the surface and the fusion of early microcolonies (Haagensen et al., 2015). These observations illustrate the improvement of community fitness through the active spatial structuration of its individuals and theirs interactions, and thereby the stabilization of their symbiotic relations.

Similar observations were made by describing the evolution of communities derived from a clonal *Burkholderia cenocepacia* biofilms (Poltak and Cooper, 2011). The authors highlighted the emergence of three variants and their persistence in mixed communities displaying enhanced productivity than any monoculture. The authors demonstrated that such productivity gains were due to the asymmetrical cross-feeding between the different ecotypes and the expansion and restructuration of biofilm space that constructed new niches. Overall, the fitness of cooperative or competitive phenotypes largely depends on neighboring cells that finally influences the spatial arrangement of genotypes within biofilms (Nadell et al., 2016; Stubbendieck et al., 2016). Reciprocally, the spatial structuring of genotypes within biofilm greatly influences the evolution of social phenotypes (Nadell et al., 2016). Many social phenotypes are regulated by QS through the secretion of diffusible signaling peptide (Nadell et al., 2016; Perchat et al., 2016). Studies showed how interspecies QS may have a role in competition interactions. In a *P. putida–P. aeruginosa* mixed-species biofilm, it was demonstrated a spatial repulsion between the two isolates (Fernandez-Piñar et al., 2011; Bridier et al., 2014). Indeed, both populations secreted molecules which negatively alter the growth of each other; *P. aeruginosa* secreted quinolone, a QS signaling molecule which inhibits biofilm formation of *P. putida*, and in the same way, *P. putida* secreted putisolvin which is regulated by QS and inhibits *P. aeruginosa* biofilm formation (Diggle et al., 2003; Kuiper et al., 2004; Fernandez-Piñar et al., 2011; Bridier et al., 2014). Other systems can have an important role in interspecies interactions such as communication and transport including outer membrane vesicles (OMVs) (Wang et al., 2015). OMVs could promote bacterial interactions and thereby participate to the architectural integrity of biofilms (Schwechheimer and Kuehn, 2015). In *Helicobacter pylori*, *Franciscella*, *P. aeruginosa*, *V. cholera* and *P. putida*, vesicles are involved in biofilm formation by increasing hydrophobicity of cells surface and by participating to the matrix formation (Yonezawa et al., 2011; Baumgarten et al., 2012; van Hoek, 2013; Altindis et al., 2014; Murphy et al., 2014; Wang et al., 2015). OMVs can also have an interspecies interference property in biofilms when they are coupled with an antimicrobial action and alter bacteria in biofilms (Schooling and Beveridge, 2006). Species interactions contribute thus through various way to shape biofilm architecture. Actually, processes related to intra-species interactions, as for instance cell death, can also play a key role in biofilm structuring. Localized cell death is known to trigger wrinkle formation of biofilm by focusing mechanical forces and instigate vertical extending of the biofilm (Asally et al., 2012; Rendueles et al., 2014; Nadell et al., 2016). Overall, it has been showed that cell death plays an important role in the development of multicellular biofilms and the subsequent dispersal of surviving cells (Webb et al., 2003; Mai-Prochnow et al., 2004). In *Bacillus subtilis* biofilms, subpopulations of cells use a cannibalistic strategy involving the production and secretion of two toxins to lyse sensitive siblings which then provide nutrients for the cannibals. Interestingly, cannibal cells correspond to the subpopulation producing the extracellular matrix, the production of toxins and matrix being triggered by surfactin, a paracrine signal whose production is controlled by the QS signaling peptide ComX (Lopez et al., 2009). This process finally promotes matrix producer subpopulations and enables the development of biofilm structure through an increase of matrix production.

This extraordinary large diversity of means provides to microorganisms the ability to dynamically shape biofilm architecture and functions using complementary mechanisms.

Numbers of processes involved in architecture plasticity are thus inter-related through complex regulation networks enabling the targeted adaptation through the sensing of a wide range of environmental conditions.

Tuning Biofilms Architecture to Control Their Functions?

Sculpting biofilm spatial organization represents an attractive approach to control their overall functions, whether beneficial or deleterious (**Table 1**). The structure of those surface-associated communities can be faceted by governing their local environmental or by exposing them to molecular and biological effectors. Illustrations of such shaping are displayed in **Figure 3**.

Manipulating Biofilm Local Environment

Within a biofilm, individual cells have the ability to monitor their direct environment (nutrients, pH, ionic strength, oxygen, surface. . .). The integration of these various external signals leads to specific cellular responses that can be exploited to alter the community structure/function.

In this line, Sauer et al. (2004) elegantly demonstrated that a sudden increase in carbon substrate or pH of the growing medium lead to significant change in *P. aeruginosa* biofilm structure. Changing the glutamate concentration of the media from 2 to 20 mM triggers a total loss of the biofilm tridimensional structure in less than 60 min. This massive loss of surface-associated biomass observed was correlated with the induction of a subpopulation of bacteria with an increased expression of flagella and a decreased expression of pilus, allowing their dispersal in the flow. Similarly, *Staphylococcus epidermidis* biofilm exposed to a high osmotic pressure (from 86 to 776 mM NaCl) decreased the average bacterial local number density by 10-fold (Stewart et al., 2013). Increasing the flow shear stress applied on *P. aeruginosa* biofilm reduced the formation of self-aggregating clusters, in particular through a significant down regulation of genes involved in extracellular polysaccharide synthesis (Crabbé et al., 2008; Dingemans et al., 2016). Exposing a gravity sewer biofilm to increasing shear stress (from 1.12 to 1.45 mPa) affected porosity of the biostructure (from 70 down to 55%) and reduced the chemical oxygen demand in the sewers from 40 to 32% (Xu et al., 2017). Growing the microaerophilic human pathogen *Campylobacter jejuni* under aerobic condition (20% O_2) stimulates the kinetic of biofilm development (Reuter et al., 2010) and the complexity in their architecture (Turonova et al., 2015). Desiccation of the biofilm occurs periodically in various environments including soils, industrial surfaces or hypersaline ponds (Habimana et al., 2014; Decho, 2016; Lennon and Lehmkuhl, 2016). In the latter environment, the EPS attains a glass state upon extreme desiccation that presumably protects the biofilm inhabitants and allows them to resume activities upon rehydratation. When grown at the air interface, *Bacillus subtilis* developed a biofilm protected by a hydrophobic raincoat layer formed by the BslA surface-active protein (Arnaouteli et al., 2016). This interfacial layer of water-repellent proteins also protects the biofilm inhabitants from ethanol and biocide action (Epstein et al., 2011). When the biofilm structure limits antimicrobial penetration and prevents the contact with the microbial target, exposition to pulsating waves of energy (e.g., ultrasonic waves) can amplify the antimicrobial effect. This so called bio-acoustic effect is likely associate with a deformation of the biofilm and a better penetration in the EPS of the toxic compounds (Qian et al., 1996; Peterson et al., 2015). Another often neglected environmental parameter to shape biofilm is the nature of the substratum. Greene et al. (2016) demonstrated that it was possible to alter the biofilm structure of *Acinetobacter baumannii* only by changing its carrier nature. While an important structured biofilm was able to grow in 4 days on polycarbonate coupons, only sparse adhering cells were visible in the same condition on glass (biofilm biovolume decreased from more than 2.5 to below 0.1 $\mu m^3/\mu m^2$). Not only the spatial arrangement of the cell were altered by the nature of the substratum, but also the bacterial physiology as reported by the live/dead ratio that ranged from less than 2 for biofilm grown on rubber to almost 8 for cell grown on stainless steel. Muszanska et al. (2012) demonstrated that coating silicone rubber with a brush polymer alters the biofilm structure (including a strong decrease in the polysaccharidic matrix) and the susceptibility to the gentamycin antibiotic. From those observations, authors suggested that the antimicrobial treatments of biofilm-associated infections could be more effective on material protected with such active antibiofilm coatings. Similarly, Valle et al. (2006) observed that treating abiotic surfaces with group II capsular polysaccharides drastically reduces both initial adhesion and biofilm architecture by important nosocomial pathogens.

All these examples illustrate the possibility to manipulate the structure/function association of microbial biofilms by controlling one (or a combination) of parameter(s) in their local environment.

Reprogramming Biofilm Structure/Function with Specific Molecular Triggers

External cues can be put in used to act both directly on the biofilm EPS properties or reprogram individual cell physiology and transcriptional expression patterns. A large palette of exogeneous enzymes has the ability to degrade specific moieties of the complex biofilm matrix. Those EPS-degrading enzymes can act specifically on extracellular polysaccharides (dispersin B), proteins (proteinase K, trypsin) or eDNA (DNase I) (Boles and Horswill, 2011). Cocktails of such enzymes are proposed in the food-industry to target persistent deleterious biofilms (Lequette et al., 2010; Nguyen and Burrows, 2014). Dispersin B that hydrolyzes the glycosidic linkages of PNAG was found to be efficient in a range of pathogenic bacteria and is being commercially developed as a wound care gel (Kaplan et al., 2003). Enzymes from bacteriophages can dissolve extracellular polysaccharides of the matrix and reverse the biofilm tolerance to antibiotics and other antimicrobial treatments (Chan and Abedon, 2015). Bacteriophage enzymes were able to reduce the alginate EPS viscosity by up to 40% in *P. aeruginosa* biofilm (Hanlon et al., 2001). Using purified EPS depolymerase isolated

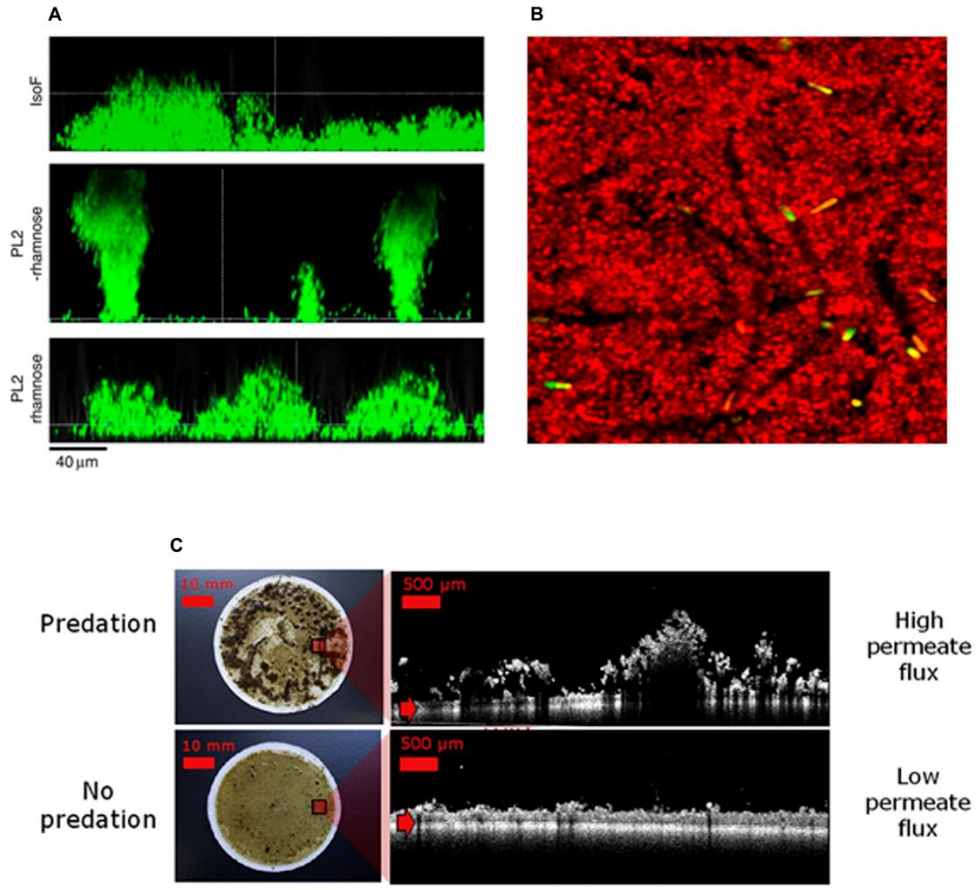

FIGURE 3 | Sculpting biofilm architecture to control their function. **(A)** Role of biosurfactants in *Pseudomonas putida* biofilm architecture. Images displayed vertical sections of biofilms grew in flow chamber during 3 days in presence or absence of rhamnoses. IsoF correspond to the wild-type strain and PL2 strain to a conditional mutant in which the native promoter region of psoA (a gene coding a large non-ribosomal peptide synthethase which directs the biosynthesis of the two cyclic lipopeptide biosurfactants putisolvin I and II) has been replaced with the rhamnose-inducible PrhaB promoter (adapted from Carcamo-Oyarce et al., 2015). Addition of 0.2% rhamnose in growth medium of PL2 lead to the recovery of the flat wild-type biofilm structure suggesting that putisolvins promote the colonization of the substratum. **(B)** Predation by protozoa affects biofilms spatial organization during gravity-driven dead-end ultrafiltration and induces higher permeate fluxes (adapted from Derlon et al., 2012). **(C)** Green bacilli creates transient pores in the biofilm matrix of *Staphylococcus aureus*, leading to an increased sensitivity to biocide action as described in Houry et al. (2012) (courtesy of Julien Deschamps, INRA).

from an *Enterobacter agglomerans* bacteriophage, Skillman et al. (1998) demonstrated a change in the physical properties of the EPS from a two species biofilms resulting in the effective removal of both species. Another telling example is the use of the biosynthetic glycoside hydrolases PelAh and PslGh that were able to disrupt the spatial organization of a pre-existing *P. aeruginosa* biofilm within 1 h, potentiating the action of colistin and neutrophils (Baker et al., 2016). By targeting the cell wall, the hydrolases LySMP was able to reduce the biofilm structure of *Streptococcus suis* by more than 80% and facilitate the action of several antibiotics on sessile communities (Meng et al., 2011).

Amyloids fibers are the "neglected child" of the EPS matrix (Dueholm and Nielsen, 2016) while evidence is rinsing that those proteinous assemblages are important drivers of the matrix viscoelastic properties (Lembré et al., 2014). D-amino acids (with some controversy) and parthenolide were identified as molecular inhibitors targeting the polymerisation or anchorage to the cell wall of TasA, the main *Bacillus subtilis* EPS amyloid (Kolodkin-Gal et al., 2010; Leiman et al., 2013; Romero et al., 2013). *P. aeruginosa* produces *cis*-2-decenoic acid, a small messenger molecule responsible for the induction of the biofilm dispersion response in a range of Gram-negative and Gram-positive bacteria. It has been shown to alter biofilm structure and to reverse tolerance to conventional antimicrobial agents (Marques et al., 2015). The matrix reprogrammation can also be triggered by biofilm cells exposition to sublethal concentration of antimicrobials. Schilcher et al. (2016) observed that subinhibitory

concentrations of clindamycin upregulated the expression of major biofilm-associated genes in *S. aureus* biofilm and shift the composition of the biofilm matrix toward higher eDNA content.

In addition to soluble molecular effectors, microorganisms are able to respond to organic and inorganic volatiles in their local headspace, some of which influencing their ability to form biofilm (Audrain et al., 2015). Nitric oxide (NO) is a volatile messenger able to trigger biofilm dispersion. Barraud et al. (2009) demonstrated that exposing a multispecies biofilms in water system to 500 nM sodium nitroprusside (NO donor) almost totally abolished the biofilm spatial organization, increasing by 20 the efficacy of the conventional chlorine treatment. On the opposite, ammonia, a volatile produced by many bacteria, stimulates biofilm formation in *Bacillus licheniformis* and other relatives (Nijland and Burgess, 2010). Similarly, it was showed that self-produced acetic acid was used as volatile signals to stimulate and coordinate the timing of biofilm formation in *B. subtilis* (Chen et al., 2015). This behavioral biofilm response triggered by odorant molecules was compared to olfaction; it opens doors to new biofilm control strategies based on airborne volatile metabolites.

Guided Biofilm Ecology to Shape the Biofilm Structures and Functions

As mentioned previously, biofilm architecture and functions are intimately related to their microbial content and the spatial repartition of their inhabitants. In, several fields including health, agriculture, food processing and environment, new strategies emerged to manipulate biofilm functions by guided biofilm ecology. The effectors of these approaches are selected organisms that can alter population structures in the targeted community such as bacteria, bacteriophages, molds, yeasts, microalgae, amoeba, and metazoans.

A family of microbial probiotics are put in used on the market to combat human biofilm-associated infections (Vuotto et al., 2014). Specific inhabitants of the oral microbiome such as *Porphyromonas gingivalis* are responsible of the production of unpleasant malodorant volatile sulfur compounds (halitosis) (Lee and Baek, 2014). Different reports described a beneficial long term effect of combining conventional oral mouthwashes chemical pretreatment with probiotic therapies involving lactic acid bacteria such as the bacteriocin producing *Streptococcus salivarius* K12 (Masdea et al., 2012; Jamali et al., 2016). Using an agent-based spatially explicit model approach, Bucci et al. (2011) demonstrated that the competitive dynamic of bacteriocin producing strain in a multispecies biofilm strongly depends on a single critical bacteriocin-range parameter that measures the threshold distance from a focal bacteriocin-producing cell whose fitness is higher than that of sensitive cell. Similarly, the biofilm of *Aggregatibacter actinomycetemcomitans* involved in chronic periodontal diseases was degraded after exposition to a Lactobacillus probiotic altering the biofilm structure (Jaffar et al., 2016). *Lactobacillus rhamnosus* GG and *Lactococcus lactis* HY449 both affect the spatial organization of model oral biofilms and reduced the count of oral pathogens in the community (Jiang et al., 2016; Kim and Lee, 2016). The most widespread use of probiotic is the treatment of gastrointestinal diseases. Rieu et al. (2014) demonstrated that a Lactobacillus-induced host immunomodulation response was strongly enhanced when the potential probiotic was cultivated as a structured biofilm in contrast with free-cells. This fundamental discovery leads to the exploration of new biofilm-based formulations to increase their *in vivo* beneficial effects (Cheow et al., 2014).

In the medical area, an emerging research field to overcome bacterial antibioresistance (super bugs) and chronic biofilm-associated infections (BAI) is the bacteriophage therapy (Maura and Debarbieux, 2011; Chan and Abedon, 2015). In the lab, bacteriophages were efficient in mice models to treat a (biofilm associated) *P. aeruginosa* acute lung infection (Debarbieux et al., 2010). Exposing *Clostridium difficile* colony biofilms to a cocktail of selected phages lead to the emergence of lysed zones and elongated cells morphotypes in the structure, but the loss of cell viability observed in early stages decreased with biofilm age (Nale et al., 2016). While highly effective on free-cells, the architecture of biofilm, the diversity of cell types and the presence of matrix likely limit the phages efficacy to treat chronic BAI. Only few human phase II trials explored this approach to treat human patient with only mitigated success (Wright et al., 2009). There is a clear need for larger scale trials and deeper research on phage and biofilm interactions in this promising emerging field (Servick, 2016).

From farms to forks, the microbiological control of raw and processed food through the food chain is still mainly ensure by the use of chemical products including pesticides, antibiotics or disinfectants. Their massive use raised some important environmental and health concerns and stressed out the need for alternative sustainable approaches. In the crop field, a recent paper pinpointed the biofilm mode of life as an important driver of the efficacy of microbial biocontrol agents (Pandin et al., 2017). Indeed, different studies showed evidence that biocontrol agent are able to form protective biofilms on crop that develop antagonistic properties against unwanted microorganisms (Zeriouh et al., 2014). The associated mechanisms likely involved many of the biofilm traits, including spatial competition, cell-cell signaling and the production of antimicrobials (Zhou et al., 2016). Spraying antagonistic *Bacillus subtilis* TSK1-1 or *Bacillus amyloliquefaciens* WG6-14 on citrus leaf surface alter the spatial organization and the density of *Xanthomonas axonopodis* pv. *citri*, a pathogenic bacteria involved in citrus canker (Huang et al., 2012). A comparative transcriptome analysis of the biocontrol agent *Bacillus amyloliquefaciens* FZB42 as response to biofilm formation showed an up regulation of the *lci* gene encoding an antimicrobial peptide, and of operons involved in the production of the extracellular matrix (Kröber et al., 2016). It was also shown that the architecture of those protective biofilm can be stimulated by plant metabolites such as root exudates (Espinosa-Urgel et al., 2002). Similar protecting biofilms are envisioned in the feed/food environments to protect livestock building and the surface of food processing equipments from pathogen persistence (Mariani et al., 2011; Piard and Briandet, 2016). Habimana et al. (2011)

demonstrated using confocal imaging and a simplified individual based model that exposing sessile cells of *Listeria monocytogenes* to *Lactococcus lactis* engaged a spatial race to interfacial nutrients resulting in a total loss of the pathogen multiplication. It was also recently shown that motile bacilli can create transient pores in *Staphylococcus aureus* biofilms, sensitizing the pathogenic structure to biocide action (Houry et al., 2012).

The use of organisms to shape new biofilm functions is also emerging in environmental sciences. Derlon and his collaborators nicely demonstrated that predation mediated by added protozoa (*Tetrahymena pyriformis*) triggers strong architectural change of an ultrafiltration membrane biofilm (from flat to heterogeneous and porous structure), increasing by 2 the permeate flux (Derlon et al., 2012). The same group also demonstrated that metazoan worms, including the nematode *Plectus aequalis* and the oligochaetes *Aelosoma hemprichi*, were also able to remodel membrane fouling biofilm structure and to increase significantly the membrane efficacy (Derlon et al., 2013; Klein et al., 2016).

The microflora of stone monuments is mainly composed of microbial biofilms and lichens. Scientists of this field implicate these complex ecosystems in stone damage while others pinpointed their bioprotective role (Pinna, 2014). Application of biofilm-induced calcium carbonate precipitation is an emerging tool for the bioremineralisation of stone and cultural heritage (Dhami et al., 2014). Dick et al. (2006) evaluated the performance of *Bacillus sphaericus* biofilms to restore deteriorated Euville limestone, a stone used for building and sculpturing in France. They demonstrated an important surface colonization and the presence of dense calcium carbonate crystals on biofilms formed on the treated stone. Similar biocalcifying effect was observed with *Bacillus subtilis* on deteriorated globigerina limestone (Micallef et al., 2016).

Environmental biofilms are largely involved in global biogeochemical cycles (Singer et al., 2010). Through human intensive activities and the resulting environmental changes, we are unintentionally affecting and remodeling those natural ecosystems. At the Paris climate conference (COP21) in 2015, 195 countries adopted a legally binding global climate deal. The agreement sets out a global action plan to put the world on track to avoid dangerous climate change by limiting global warming to well below 2°C above pre-industrial levels (Rhodes, 2016). Indeed, a 2°C warming in flowing water is already enough to drive significant changes in freshwater biofilm structure/function by inducing a complex reorganization in the network of interactions among microbial populations within the biofilm matrix (Romani et al., 2014).

CONCLUSION

Architectural plasticity of biofilm constitutes a central process to actively adapt to stress and to increase productivity and fitness of microbial communities in response to changing environmental conditions. Considering dynamics of biofilm structure is thus required to better understand the emergence of novel functional properties and to decipher the communal mechanisms underlying microbial behavior, from single cell to multicellular community. Although our ability to predict and manage the functional properties and adaptation strategies of these complex dynamic communities is yet limited, the increasing development of predictive modeling approaches and the improvement of integration of experiments and models should, in a near future, enable to better link composition, dynamic organization and function of microbial communities (Widder et al., 2016). Recent technological advances in single-cell analytic methods have led to the generation of quantities of novel interesting data on individual microbial behaviors which still are to be exploited through individual-based modeling approach for instance, to provide insights into self-organized spatial patterns and to construct a realistic vision of biofilm at both the individual and community levels (Hellweger et al., 2016).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

CP is supported by a grant from Région Ile-de-France, DIM ASTREA. We warmly acknowledge A. Canette from the MIMA2 microscopy platform and J. Deschamps (INRA) for biofilm imaging.

REFERENCES

Abdallah, M., Khelissa, O., Ibrahim, A., Benoliel, C., Heliot, L., Dhulster, P., et al. (2015). Impact of growth temperature and surface type on the resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms to disinfectants. *Int. J. Food Microbiol.* 214, 38–47. doi: 10.1016/j.ijfoodmicro.2015.07.022

Ahmad, I., Wigren, E., Le Guyon, S., Vekkeli, S., Blanka, A., El Mouali, Y., et al. (2013). The EAL-like protein STM1697 regulates virulence phenotypes, motility and biofilm formation in *Salmonella typhimurium*. *Mol. Microbiol.* 90, 1216–1232. doi: 10.1111/mmi.12428

Altindis, E., Fu, Y., and Mekalanos, J. J. (2014). Proteomic analysis of *Vibrio cholerae* outer membrane vesicles. *Proc. Natl. Acad. Sci. U.S.A.* 111, E1548–E1556. doi: 10.1073/pnas.1403683111

Arnaouteli, S., MacPhee, C. E., and Stanley-Wall, N. R. (2016). Just in case it rains: building a hydrophobic biofilm the *Bacillus subtilis* way. *Curr. Opin. Microbiol.* 34, 7–12. doi: 10.1016/j.mib.2016.07.012

Asally, M., Kittisopikul, M., Rue, P., Du, Y., Hu, Z., Cagatay, T., et al. (2012). Localized cell death focuses mechanical forces during 3D patterning in a biofilm. *Proc. Natl. Acad. Sci. U.S.A.* 109, 18891–18896. doi: 10.1073/pnas.1212429109

Audrain, B., Farag, M. A., Ryu, C. M., and Ghigo, J. M. (2015). Role of bacterial volatile compounds in bacterial biology. *FEMS Microbiol. Rev.* 39, 222–233. doi: 10.1093/femsre/fuu013

Azeredo, J., Azevedo, N. F., Briandet, R., Cerca, N., Coenye, T., Costa, A. R., et al. (2017). Critical review on biofilm methods. *Crit. Rev. Microbiol.* 43, 313–351. doi: 10.1080/1040841X.2016.1208146

Baker, P., Hill, P. J., Snarr, B. D., Alnabelseya, N., Pestrak, M. J., Lee, M. J., et al. (2016). Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent *Pseudomonas aeruginosa* biofilms. *Sci. Adv.* 2:e1501632. doi: 10.1126/sciadv.1501632

Barraud, N., Schleheck, D., Klebensberger, J., Webb, J. S., Hassett, D. J., Rice, S. A., et al. (2009). Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. *J. Bacteriol.* 191, 7333–7342. doi: 10.1128/JB.00975-09

Battin, T. J., Besemer, K., Bengtsson, M. M., Romani, A. M., and Packmann, A. I. (2016). The ecology and biogeochemistry of stream biofilms. *Nat. Rev. Microbiol.* 14, 251–263. doi: 10.1038/nrmicro.2016.15

Baumgarten, T., Sperling, S., Seifert, J., von Bergen, M., Steiniger, F., Wick, L. Y., et al. (2012). Membrane vesicle formation as a multiple-stress response mechanism enhances *Pseudomonas* putida DOT-T1E cell surface hydrophobicity and biofilm formation. *Appl. Environ. Microbiol.* 78, 6217–6224. doi: 10.1128/AEM.01525-12

Beech, I. B., and Sunner, J. (2004). Biocorrosion: towards understanding interactions between biofilms and metals. *Curr. Opin. Biotechnol.* 15, 181–186. doi: 10.1016/j.copbio.2004.05.001

Berlanga, M., and Guerrero, R. (2016). Living together in biofilms: the microbial cell factory and its biotechnological implications. *Microb. Cell Fact.* 15:165. doi: 10.1186/s12934-016-0569-5

Berleman, J. E., Zemla, M., Remis, J. P., Liu, H., Davis, A. E., Worth, A. N., et al. (2016). Exopolysaccharide microchannels direct bacterial motility and organize multicellular behavior. *ISME J.* 10, 2620–2632. doi: 10.1038/ismej.2016.60

Besemer, K., Hodl, I., Singer, G., and Battin, T. J. (2009). Architectural differentiation reflects bacterial community structure in stream biofilms. *ISME J.* 3, 1318–1324. doi: 10.1038/ismej.2009.73

Bjarnsholt, T. (2013). The role of bacterial biofilms in chronic infections. *APMIS Suppl.* 136, 1–51. doi: 10.1111/apm.12099

Boles, B. R., and Horswill, A. R. (2011). Staphylococcal biofilm disassembly. *Trends Microbiol.* 19, 449–455. doi: 10.1016/j.tim.2011.06.004

Boles, B. R., Thoendel, M., and Singh, P. K. (2004). Self-generated diversity produces "insurance effects" in biofilm communities. *Proc. Natl. Acad. Sci. U.S.A.* 101, 16630–16635. doi: 10.1073/pnas.0407460101

Bridier, A., Briandet, R., Bouchez, T., and Jabot, F. (2014). A model-based approach to detect interspecific interactions during biofilm development. *Biofouling* 30, 761–771. doi: 10.1080/08927014.2014.923409

Bridier, A., Briandet, R., Thomas, V., and Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: a review. *Biofouling* 27, 1017–1032. doi: 10.1080/08927014.2011.626899

Bucci, V., Nadell, C. D., and Xavier, J. B. (2011). The evolution of bacteriocin production in bacterial biofilms. *Am. Nat.* 178, E162–E173. doi: 10.1086/662668

Carcamo-Oyarce, G., Lumjiaktase, P., Kummerli, R., and Eberl, L. (2015). Quorum sensing triggers the stochastic escape of individual cells from *Pseudomonas putida* biofilms. *Nat. Commun.* 6:5945. doi: 10.1038/ncomms6945

Chambers, J. R., and Sauer, K. (2013). Small RNAs and their role in biofilm formation. *Trends Microbiol.* 21, 39–49. doi: 10.1016/j.tim.2012.10.008

Chan, B. K., and Abedon, S. T. (2015). Bacteriophages and their enzymes in biofilm control. *Curr. Pharm. Des.* 21, 85–99. doi: 10.2174/1381612820666140905112311

Chen, Y., Gozzi, K., Yan, F., and Chai, Y. (2015). Acetic acid acts as a volatile signal to stimulate bacterial biofilm formation. *mBio* 6:e00392. doi: 10.1128/mBio.00392-15

Cheow, W. S., Kiew, T. Y., and Hadinoto, K. (2014). Controlled release of Lactobacillus rhamnosus biofilm probiotics from alginate-locust bean gum microcapsules. *Carbohydr. Polym.* 103, 587–595. doi: 10.1016/j.carbpol.2014.01.036

Conibear, T. C., Collins, S. L., and Webb, J. S. (2009). Role of mutation in *Pseudomonas aeruginosa* biofilm development. *PLoS ONE* 4:e6289. doi: 10.1371/journal.pone.0006289

Crabbé, A., De Boever, P., Van Houdt, R., Moors, H., Mergeay, M., and Cornelis, P. (2008). Use of the rotating wall vessel technology to study the effect of shear stress on growth behaviour of *Pseudomonas aeruginosa* PA01. *Environ. Microbiol.* 10, 2098–2110. doi: 10.1111/j.1462-2920.2008.01631.x

Debarbieux, L., Leduc, D., Maura, D., Morello, E., Criscuolo, A., Grossi, O., et al. (2010). Bacteriophages can treat and prevent *Pseudomonas aeruginosa* lung infections. *J. Infect. Dis.* 201, 1096–1104. doi: 10.1086/651135

Decho, A. (2016). "Unique and baffling aspects of the matrix: EPS syneresis and glass formation during desiccation," in *The Perfect Slime, Microbial extracellular Polymeric Substances (EPS)*, eds H. C. Flemming, T. R. Neu, and J. Wingender (London: IWA Publishing), 207–226.

Derlon, N., Koch, N., Eugster, B., Posch, T., Pernthaler, J., Pronk, W., et al. (2013). Activity of metazoa governs biofilm structure formation and enhances permeate flux during Gravity-Driven Membrane (GDM) filtration. *Water Res.* 47, 2085–2095. doi: 10.1016/j.watres.2013.01.033

Derlon, N., Peter-Varbanets, M., Scheidegger, A., Pronk, W., and Morgenroth, E. (2012). Predation influences the structure of biofilm developed on ultrafiltration membranes. *Water Res.* 46, 3323–3333. doi: 10.1016/j.watres.2012.03.031

Dhami, N. K., Reddy, M. S., and Mukherjee, A. (2014). Application of calcifying bacteria for remediation of stones and cultural heritages. *Front. Microbiol.* 5:304. doi: 10.3389/fmicb.2014.00304

Dick, J., De Windt, W., De Graef, B., Saveyn, H., Van der Meeren, P., De Belie, N., et al. (2006). Bio-deposition of a calcium carbonate layer on degraded limestone by *Bacillus* species. *Biodegradation* 17, 357–367. doi: 10.1007/s10532-005-9006-x

Diggle, S. P., Winzer, K., Chhabra, S. R., Worrall, K. E., Camara, M., and Williams, P. (2003). The *Pseudomonas aeruginosa* quinolone signal molecule overcomes the cell density-dependency of the quorum sensing hierarchy, regulates rhl-dependent genes at the onset of stationary phase and can be produced in the absence of LasR. *Mol. Microbiol.* 50, 29–43. doi: 10.1046/j.1365-2958.2003.03672.x

Dingemans, J., Monsieurs, P., Yu, S. H., Crabbe, A., Forstner, K. U., Malfroot, A., et al. (2016). Effect of shear stress on *Pseudomonas aeruginosa* isolated from the cystic fibrosis lung. *mBio* 7:e00813-16. doi: 10.1128/mBio.00813-16

Donelli, G., Francolini, I., Romoli, D., Guaglianone, E., Piozzi, A., Ragunath, C., et al. (2007). Synergistic activity of dispersin B and cefamandole nafate in inhibition of staphylococcal biofilm growth on polyurethanes. *Antimicrob. Agents Chemother.* 51, 2733–2740. doi: 10.1128/AAC.01249-06

Drescher, K., Dunkel, J., Nadell, C. D., van Teeffelen, S., Grnja, I., Wingreen, N. S., et al. (2016). Architectural transitions in *Vibrio cholerae* biofilms at single-cell resolution. *Proc. Natl. Acad. Sci. U.S.A.* 113, E2066–E2072. doi: 10.1073/pnas.1601702113

Dubois-Brissonnet, F., Trotier, E., and Briandet, R. (2016). The biofilm lifestyle involves an increase in bacterial membrane saturated fatty acids. *Front. Microbiol.* 7:1673. doi: 10.3389/fmicb.2016.01673

Dueholm, M. S., and Nielsen, P. H. (2016). "Amyloids - a neglected child of the slime," in *The Perfect Slime, Microbial Extracellular Polymeric Substances (EPS)*, eds H. C. Flemming, T. R. Neu, and J. Wingender (London: IWA Publishing), 113–133.

Elsholz, A. K., Wacker, S. A., and Losick, R. (2014). Self-regulation of exopolysaccharide production in *Bacillus subtilis* by a tyrosine kinase. *Genes Dev.* 28, 1710–1720. doi: 10.1101/gad.246397.114

Epstein, A. K., Pokroy, B., Seminara, A., and Aizenberg, J. (2011). Bacterial biofilm shows persistent resistance to liquid wetting and gas penetration. *Proc. Natl. Acad. Sci. U.S.A.* 108, 995–1000. doi: 10.1073/pnas.1011033108

Espinosa-Urgel, M., Kolter, R., and Ramos, J. L. (2002). Root colonization by *Pseudomonas* putida: love at first sight. *Microbiology* 148(Pt 2), 341–343. doi: 10.1099/00221287-148-2-341

Feliziani, S., Lujan, A. M., Moyano, A. J., Sola, C., Bocco, J. L., Montanaro, P., et al. (2010). Mucoidy, quorum sensing, mismatch repair and antibiotic resistance in *Pseudomonas aeruginosa* from cystic fibrosis chronic airways infections. *PLoS ONE* 5:e12669. doi: 10.1371/journal.pone.0012669

Fernandez-Piñar, R., Camara, M., Dubern, J. F., Ramos, J. L., and Espinosa-Urgel, M. (2011). The *Pseudomonas aeruginosa* quinolone quorum sensing signal alters the multicellular behaviour of *Pseudomonas* putida KT2440. *Res. Microbiol.* 162, 773–781. doi: 10.1016/j.resmic.2011.06.013

Finnegan, S., and Percival, S. L. (2015). EDTA: an antimicrobial and antibiofilm agent for use in wound care. *Adv. Wound Care* 4, 415–421. doi: 10.1089/wound.2014.0577

Flemming, H. C. (2011). The perfect slime. *Colloids Surf. B Biointerfaces* 86, 251–259. doi: 10.1016/j.colsurfb.2011.04.025

Flemming, H. C., Neu, T. R., and Wozniak, D. J. (2007). The EPS matrix: the "house of biofilm cells". *J. Bacteriol.* 189, 7945–7947. doi: 10.1128/JB.00858-07

Flemming, H. C., and Wingender, J. (2010). The biofilm matrix. *Nat. Rev. Microbiol.* 8, 623–633. doi: 10.1038/nrmicro2415

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* 14, 563–575. doi: 10.1038/nrmicro.2016.94

Fong, J. N., and Yildiz, F. H. (2015). Biofilm matrix proteins. *Microbiol. Spectr.* 3:MB-0004-2014. doi: 10.1128/microbiolspec.MB-0004-2014

Giaouris, E., Chorianopoulos, N., Doulgeraki, A., and Nychas, G. J. (2013). Co-culture with *Listeria monocytogenes* within a dual-species biofilm community strongly increases resistance of *Pseudomonas putida* to benzalkonium chloride. *PLoS ONE* 8:e77276. doi: 10.1371/journal.pone.0077276

Giaouris, E., Heir, E., Desvaux, M., Hebraud, M., Moretro, T., Langsrud, S., et al. (2015). Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. *Front. Microbiol.* 6:841. doi: 10.3389/fmicb.2015.00841

Greene, C., Wu, J., Rickard, A. H., and Xi, C. (2016). Evaluation of the ability of *Acinetobacter baumannii* to form biofilms on six different biomedical relevant surfaces. *Lett. Appl. Microbiol.* 63, 233–239. doi: 10.1111/lam.12627

Guilhen, C., Charbonnel, N., Parisot, N., Gueguen, N., Iltis, A., Forestier, C., et al. (2016). Transcriptional profiling of *Klebsiella pneumoniae* defines signatures for planktonic, sessile and biofilm-dispersed cells. *BMC Genomics* 17:237. doi: 10.1186/s12864-016-2557-x

Gutiérrez, O., Juan, C., Pérez, J. L., and Oliver, A. (2004). Lack of association between hypermutation and antibiotic resistance development in *Pseudomonas aeruginosa* isolates from intensive care unit patients. *Antimicrob. Agents Chemother.* 48, 3573–3575. doi: 10.1128/AAC.48.9.3573-3575.2004

Guttenplan, S. B., Blair, K. M., and Kearns, D. B. (2010). The EpsE flagellar clutch is bifunctional and synergizes with EPS biosynthesis to promote *Bacillus subtilis* biofilm formation. *PLoS Genet.* 6:e1001243. doi: 10.1371/journal.pgen.1001243

Guttenplan, S. B., and Kearns, D. B. (2013). Regulation of flagellar motility during biofilm formation. *FEMS Microbiol. Rev.* 37, 849–871. doi: 10.1111/1574-6976.12018

Haagensen, J. A., Hansen, S. K., Christensen, B. B., Pamp, S. J., and Molin, S. (2015). Development of spatial distribution patterns by biofilm cells. *Appl. Environ. Microbiol.* 81, 6120–6128. doi: 10.1128/AEM.01614-15

Habimana, O., Guillier, L., Kulakauskas, S., and Briandet, R. (2011). Spatial competition with *Lactococcus lactis* in mixed-species continuous-flow biofilms inhibits *Listeria monocytogenes* growth. *Biofouling* 27, 1065–1072. doi: 10.1080/08927014.2011.626124

Habimana, O., Nesse, L. L., Moretro, T., Berg, K., Heir, E., Vestby, L. K., et al. (2014). The persistence of *Salmonella* following desiccation under feed processing environmental conditions: a subject of relevance. *Lett. Appl. Microbiol.* 59, 464–470. doi: 10.1111/lam.12308

Hanlon, G. W., Denyer, S. P., Olliff, C. J., and Ibrahim, L. J. (2001). Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.* 67, 2746–2753. doi: 10.1128/AEM.67.6.2746-2753.2001

Hannan, S., Ready, D., Jasni, A. S., Rogers, M., Pratten, J., and Roberts, A. P. (2010). Transfer of antibiotic resistance by transformation with eDNA within oral biofilms. *FEMS Immunol. Med. Microbiol.* 59, 345–349. doi: 10.1111/j.1574-695X.2010.00661.x

Hansen, S. K., Rainey, P. B., Haagensen, J. A., and Molin, S. (2007). Evolution of species interactions in a biofilm community. *Nature* 445, 533–536. doi: 10.1038/nature05514

Hassett, D. J., Ma, J. F., Elkins, J. G., McDermott, T. R., Ochsner, U. A., West, S. E., et al. (1999). Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Mol. Microbiol.* 34, 1082–1093. doi: 10.1046/j.1365-2958.1999.01672.x

Hellweger, F. L., Clegg, R. J., Clark, J. R., Plugge, C. M., and Kreft, J. U. (2016). Advancing microbial sciences by individual-based modelling. *Nat. Rev. Microbiol.* 14, 461–471. doi: 10.1038/nrmicro.2016.62

Hoffman, L. R., D'Argenio, D. A., MacCoss, M. J., Zhang, Z., Jones, R. A., and Miller, S. I. (2005). Aminoglycoside antibiotics induce bacterial biofilm formation. *Nature* 436, 1171–1175. doi: 10.1038/nature03912

Houry, A., Gohar, M., Deschamps, J., Tischenko, E., Aymerich, S., Gruss, A., et al. (2012). Bacterial swimmers that infiltrate and take over the biofilm matrix. *Proc. Natl. Acad. Sci. U.S.A.* 109, 13088–13093. doi: 10.1073/pnas.1200791109

Hu, W., Li, L., Sharma, S., Wang, J., McHardy, I., Lux, R., et al. (2012). DNA builds and strengthens the extracellular matrix in *Myxococcus xanthus* biofilms by interacting with exopolysaccharides. *PLoS ONE* 7:e51905. doi: 10.1371/journal.pone.0051905

Huang, T. P., Tzeng, D. D., Wong, A. C., Chen, C. H., Lu, K. M., Lee, Y. H., et al. (2012). DNA polymorphisms and biocontrol of *Bacillus* antagonistic to citrus bacterial canker with indication of the interference of phyllosphere biofilms. *PLoS ONE* 7:e42124. doi: 10.1371/journal.pone.0042124

Huynh, T. T., McDougald, D., Klebensberger, J., Al Qarni, B., Barraud, N., Rice, S. A., et al. (2012). Glucose starvation-induced dispersal of *Pseudomonas aeruginosa* biofilms is cAMP and energy dependent. *PLoS ONE* 7:e42874. doi: 10.1371/journal.pone.0042874

Jaffar, N., Ishikawa, Y., Mizuno, K., Okinaga, T., and Maeda, T. (2016). Mature biofilm degradation by potential probiotics: *Aggregatibacter actinomycetemcomitans* versus *Lactobacillus* spp. *PLoS ONE* 11:e0159466. doi: 10.1371/journal.pone.0159466

Jamali, Z., Aminabadi, N. A., Samiei, M., Sighari Deljavan, A., Shokravi, M., and Shirazi, S. (2016). Impact of chlorhexidine pretreatment followed by probiotic *Streptococcus salivarius* strain K12 on halitosis in children: a randomised controlled clinical trial. *Oral. Health Prev. Dent.* 14, 305–313. doi: 10.3290/j.ohpd.a36521

Jiang, Q., Stamatova, I., Kainulainen, V., Korpela, R., and Meurman, J. H. (2016). Interactions between *Lactobacillus rhamnosus* GG and oral micro-organisms in an in vitro biofilm model. *BMC Microbiol.* 16:149. doi: 10.1186/s12866-016-0759-7

Justice, S. S., Harrison, A., Becknell, B., and Mason, K. M. (2014). Bacterial differentiation, development, and disease: mechanisms for survival. *FEMS Microbiol. Lett.* 360, 1–8. doi: 10.1111/1574-6968.12602

Justice, S. S., Hung, C., Theriot, J. A., Fletcher, D. A., Anderson, G. G., Footer, M. J., et al. (2004). Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 101, 1333–1338. doi: 10.1073/pnas.0308125100

Justice, S. S., Hunstad, D. A., Cegelski, L., and Hultgren, S. J. (2008). Morphological plasticity as a bacterial survival strategy. *Nat. Rev. Microbiol.* 6, 162–168. doi: 10.1038/nrmicro1820

Justice, S. S., Hunstad, D. A., Seed, P. C., and Hultgren, S. J. (2006). Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19884–19889. doi: 10.1073/pnas.0606329104

Kalmokoff, M., Lanthier, P., Tremblay, T. L., Foss, M., Lau, P. C., Sanders, G., et al. (2006). Proteomic analysis of *Campylobacter jejuni* 11168 biofilms reveals a role for the motility complex in biofilm formation. *J. Bacteriol.* 188, 4312–4320. doi: 10.1128/JB.01975-05

Kaminskaya, A., Pushkareva, V., Moisenovich, M., Stepanova, T., Volkova, N., Romanova, J., et al. (2007). Stimulation of biofilm formation by insertion of Tetrahymena pyriformis wells within *Burkholderia cenocepacia* biofilms. *Mol. Genet. Microbiol. Virol.* 22, 186–194. doi: 10.3103/s0891416807040088

Kaplan, J. B., Meyenhofer, M. F., and Fine, D. H. (2003). Biofilm growth and detachment of *Actinobacillus actinomycetemcomitans*. *J. Bacteriol.* 185, 1399–1404. doi: 10.1128/JB.185.4.1399-1404.2003

Khemiri, A., Jouenne, T., and Cosette, P. (2016). Proteomics dedicated to biofilmology: what have we learned from a decade of research? *Med. Microbiol. Immunol.* 205, 1–19. doi: 10.1007/s00430-015-0423-0

Kim, Y. J., and Lee, S. H. (2016). Inhibitory effect of *Lactococcus lactis* HY 449 on cariogenic biofilm. *J. Microbiol. Biotechnol.* 26, 1829–1835. doi: 10.4014/jmb.1604.04008

Klein, T., Zihlmann, D., Derlon, N., Isaacson, C., Szivak, I., Weissbrodt, D. G., et al. (2016). Biological control of biofilms on membranes by metazoans. *Water Res.* 88, 20–29. doi: 10.1016/j.watres.2015.09.050

Koch, G., Yepes, A., Forstner, K. U., Wermser, C., Stengel, S. T., Modamio, J., et al. (2014). Evolution of resistance to a last-resort antibiotic in *Staphylococcus aureus* via bacterial competition. *Cell* 158, 1060–1071. doi: 10.1016/j.cell.2014.06.046

Kolodkin-Gal, I., Romero, D., Cao, S., Clardy, J., Kolter, R., and Losick, R. (2010). D-amino acids trigger biofilm disassembly. *Science* 328, 627–629. doi: 10.1126/science.1188628

Kostakioti, M., Hadjifrangiskou, M., and Hultgren, S. J. (2013). Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb. Perspect. Med.* 3:a010306. doi: 10.1101/cshperspect.a010306

Kragh, K. N., Hutchison, J. B., Melaugh, G., Rodesney, C., Roberts, A. E., Irie, Y., et al. (2016). Role of multicellular aggregates in biofilm formation. *mBio* 7:e00237-16. doi: 10.1128/mBio.00237-16

Kröber, M., Verwaaijen, B., Wibberg, D., Winkler, A., Puhler, A., and Schluter, A. (2016). Comparative transcriptome analysis of the biocontrol strain *Bacillus amyloliquefaciens* FZB42 as response to biofilm formation analyzed by RNA sequencing. *J. Biotechnol.* 231, 212–223. doi: 10.1016/j.jbiotec.2016.06.013

Król, J. E., Wojtowicz, A. J., Rogers, L. M., Heuer, H., Smalla, K., Krone, S. M., et al. (2013). Invasion of *E. coli* biofilms by antibiotic resistance plasmids. *Plasmid* 70, 110–119. doi: 10.1016/j.plasmid.2013.03.003

Kuiper, I., Lagendijk, E. L., Pickford, R., Derrick, J. P., Lamers, G. E., Thomas-Oates, J. E., et al. (2004). Characterization of two *Pseudomonas putida* lipopeptide biosurfactants, putisolvin I and II, which inhibit biofilm formation and break down existing biofilms. *Mol. Microbiol.* 51, 97–113. doi: 10.1046/j.1365-2958.2003.03751.x

Lee, S. H., and Baek, D. H. (2014). Effects of *Streptococcus thermophilus* on volatile sulfur compounds produced by *Porphyromonas gingivalis*. *Arch. Oral. Biol.* 59, 1205–1210. doi: 10.1016/j.archoralbio.2014.07.006

Leiman, S. A., May, J. M., Lebar, M. D., Kahne, D., Kolter, R., and Losick, R. (2013). D-amino acids indirectly inhibit biofilm formation in *Bacillus subtilis* by interfering with protein synthesis. *J. Bacteriol.* 195, 5391–5395. doi: 10.1128/JB.00975-13

Lembré, P., Di Martino, P., and Vendrely, C. (2014). Amyloid peptides derived from CsgA and FapC modify the viscoelastic properties of biofilm model matrices. *Biofouling* 30, 415–426. doi: 10.1080/08927014.2014.880112

Lennon, J. T., and Lehmkuhl, B. K. (2016). A trait-based approach to bacterial biofilms in soil. *Environ. Microbiol.* 18, 2732–2742. doi: 10.1111/1462-2920.13331

Lenz, A. P., Williamson, K. S., Pitts, B., Stewart, P. S., and Franklin, M. J. (2008). Localized gene expression in *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.* 74, 4463–4471. doi: 10.1128/AEM.00710-08

Lequette, Y., Boels, G., Clarisse, M., and Faille, C. (2010). Using enzymes to remove biofilms of bacterial isolates sampled in the food-industry. *Biofouling* 26, 421–431. doi: 10.1080/08927011003699535

Liao, S., Klein, M. I., Heim, K. P., Fan, Y., Bitoun, J. P., Ahn, S. J., et al. (2014). *Streptococcus mutans* extracellular DNA is upregulated during growth in biofilms, actively released via membrane vesicles, and influenced by components of the protein secretion machinery. *J. Bacteriol.* 196, 2355–2366. doi: 10.1128/JB.01493-14

Limoli, D., Jones, C., and Wozniak, D. (2015). "Bacterial extracellular polysaccharides in biofilm formation and function," in *Microbial Biofilms*, 2nd Edn, eds. M. Ghannoum, M. Parsek, M. Whiteley, and P. Mukherjee (Washington, DC: ASM Press), 223–247. doi: 10.1128/microbiolspec.MB-0011-2014

Liu, W., Roder, H. L., Madsen, J. S., Bjarnsholt, T., Sorensen, S. J., and Burmolle, M. (2016). Interspecific bacterial interactions are reflected in multispecies biofilm spatial organization. *Front. Microbiol.* 7:1366. doi: 10.3389/fmicb.2016.01366

Lopez, D., Vlamakis, H., Losick, R., and Kolter, R. (2009). Cannibalism enhances biofilm development in *Bacillus subtilis*. *Mol. Microbiol.* 74, 609–618. doi: 10.1111/j.1365-2958.2009.06882.x

Luján, A. M., Macia, M. D., Yang, L., Molin, S., Oliver, A., and Smania, A. M. (2011). Evolution and adaptation in *Pseudomonas aeruginosa* biofilms driven by mismatch repair system-deficient mutators. *PLoS ONE* 6:e27842. doi: 10.1371/journal.pone.0027842

Macià, M. D., Perez, J. L., Molin, S., and Oliver, A. (2011). Dynamics of mutator and antibiotic-resistant populations in a pharmacokinetic/pharmacodynamic model of *Pseudomonas aeruginosa* biofilm treatment. *Antimicrob. Agents Chemother.* 55, 5230–5237. doi: 10.1128/AAC.00617-11

Madsen, J. S., Burmolle, M., Hansen, L. H., and Sorensen, S. J. (2012). The interconnection between biofilm formation and horizontal gene transfer. *FEMS Immunol. Med. Microbiol.* 65, 183–195. doi: 10.1111/j.1574-695X.2012.00960.x

Mai-Prochnow, A., Evans, F., Dalisay-Saludes, D., Stelzer, S., Egan, S., James, S., et al. (2004). Biofilm development and cell death in the marine bacterium *Pseudoalteromonas tunicata*. *Appl. Environ. Microbiol.* 70, 3232–3238. doi: 10.1128/AEM.70.6.3232-3238.2004

Maisetta, G., Grassi, L., Di Luca, M., Bombardelli, S., Medici, C., Brancatisano, F. L., et al. (2016). Anti-biofilm properties of the antimicrobial peptide temporin 1Tb and its ability, in combination with EDTA, to eradicate *Staphylococcus epidermidis* biofilms on silicone catheters. *Biofouling* 32, 787–800. doi: 10.1080/08927014.2016.1194401

Mangalappalli-Illathu, A. K., Vidovic, S., and Korber, D. R. (2008). Differential adaptive response and survival of *Salmonella enterica* serovar enteritidis planktonic and biofilm cells exposed to benzalkonium chloride. *Antimicrob. Agents Chemother.* 52, 3669–3680. doi: 10.1128/AAC.00073-08

Mann, E. E., Rice, K. C., Boles, B. R., Endres, J. L., Ranjit, D., Chandramohan, L., et al. (2009). Modulation of eDNA release and degradation affects *Staphylococcus aureus* biofilm maturation. *PLoS ONE* 4:e5822. doi: 10.1371/journal.pone.0005822

Marchal, M., Briandet, R., Halter, D., Koechler, S., DuBow, M. S., Lett, M. C., et al. (2011). Subinhibitory arsenite concentrations lead to population dispersal in *Thiomonas* sp. *PLoS ONE* 6:e23181. doi: 10.1371/journal.pone.0023181

Mariani, C., Oulahal, N., Chamba, J. F., Dubois-Brissonnet, F., Notz, E., and Briandet, R. (2011). Inhibition of *Listeria monocytogenes* by resident biofilms present on wooden shelves used for cheese ripening. *Food Control* 22, 1357–1362. doi: 10.1016/j.foodcont.2011.02.012

Marques, C. N., Davies, D. G., and Sauer, K. (2015). Control of biofilms with the fatty acid signaling molecule cis-2-decenoic acid. *Pharmaceuticals* 8, 816–835. doi: 10.3390/ph8040816

Martin, M., Holscher, T., Dragos, A., Cooper, V. S., and Kovacs, A. T. (2016). Laboratory evolution of microbial interactions in bacterial biofilms. *J. Bacteriol.* 198, 2564–2571. doi: 10.1128/JB.01018-15

Martinez, L. C., and Vadyvaloo, V. (2014). Mechanisms of post-transcriptional gene regulation in bacterial biofilms. *Front. Cell Infect. Microbiol.* 4:38. doi: 10.3389/fcimb.2014.00038

Masdea, L., Kulik, E. M., Hauser-Gerspach, I., Ramseier, A. M., Filippi, A., and Waltimo, T. (2012). Antimicrobial activity of *Streptococcus salivarius* K12 on bacteria involved in oral malodour. *Arch. Oral. Biol.* 57, 1041–1047. doi: 10.1016/j.archoralbio.2012.02.011

Maura, D., and Debarbieux, L. (2011). Bacteriophages as twenty-first century antibacterial tools for food and medicine. *Appl. Microbiol. Biotechnol.* 90, 851–859. doi: 10.1007/s00253-011-3227-1

Meng, X., Shi, Y., Ji, W., Meng, X., Zhang, J., Wang, H., et al. (2011). Application of a bacteriophage lysin to disrupt biofilms formed by the animal pathogen *Streptococcus suis*. *Appl. Environ. Microbiol.* 77, 8272–8279. doi: 10.1128/AEM.05151-11

Micallef, R., Vella, D., Sinagra, E., and Zammit, G. (2016). Biocalcifying *Bacillus subtilis* cells effectively consolidate deteriorated *Globigerina* limestone. *J. Ind. Microbiol. Biotechnol.* 43, 941–952. doi: 10.1007/s10295-016-1768-0

Molin, S., and Tolker-Nielsen, T. (2003). Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. *Curr. Opin. Biotechnol.* 14, 255–261. doi: 10.1016/S0958-1669(03)00036-3

Monds, R. D., and O'Toole, G. A. (2009). The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol.* 17, 73–87. doi: 10.1016/j.tim.2008.11.001

Moreno-Paz, M., Gomez, M. J., Arcas, A., and Parro, V. (2010). Environmental transcriptome analysis reveals physiological differences between biofilm and planktonic modes of life of the iron oxidizing bacteria *Leptospirillum* spp. in their natural microbial community. *BMC Genomics* 11:404. doi: 10.1186/1471-2164-11-404

Murphy, K., Park, A. J., Hao, Y., Brewer, D., Lam, J. S., and Khursigara, C. M. (2014). Influence of O polysaccharides on biofilm development and outer membrane vesicle biogenesis in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* 196, 1306–1317. doi: 10.1128/JB.01463-13

Muszanska, A. K., Nejadnik, M. R., Chen, Y., van den Heuvel, E. R., Busscher, H. J., van der Mei, H. C., et al. (2012). Bacterial adhesion forces with substratum surfaces and the susceptibility of biofilms to antibiotics. *Antimicrob. Agents Chemother.* 56, 4961–4964. doi: 10.1128/AAC.00431-12

Nadell, C. D., Drescher, K., and Foster, K. R. (2016). Spatial structure, cooperation and competition in biofilms. *Nat. Rev. Microbiol.* 14, 589–600. doi: 10.1038/nrmicro.2016.84

Nadell, C. D., Foster, K. R., and Xavier, J. B. (2010). Emergence of spatial structure in cell groups and the evolution of cooperation. *PLoS Comput. Biol.* 6:e1000716. doi: 10.1371/journal.pcbi.1000716

Nadell, C. D., Xavier, J. B., and Foster, K. R. (2009). The sociobiology of biofilms. *FEMS Microbiol. Rev.* 33, 206–224. doi: 10.1111/j.1574-6976.2008.00150.x

Nale, J. Y., Chutia, M., Carr, P., Hickenbotham, P. T., and Clokie, M. R. (2016). 'Get in early'; biofilm and wax moth (*Galleria mellonella*) models reveal new insights into the therapeutic potential of *Clostridium difficile* Bacteriophages. *Front. Microbiol.* 7:1383. doi: 10.3389/fmicb.2016.01383

Neu, T. R., and Lawrence, J. R. (2016). "The extracellular matrix - an intractable part of biofilm systems," in *The Perfect Slime, Microbial Extracellular Polymeric Substances (EPS)*, eds H. C. Flemming, T. R. Neu, and J. Wingender (London: IWA Publishing), 25–60.

Nguyen, U. T., and Burrows, L. L. (2014). DNase I and proteinase K impair Listeria monocytogenes biofilm formation and induce dispersal of pre-existing biofilms. *Int. J. Food Microbiol.* 187, 26–32. doi: 10.1016/j.ijfoodmicro.2014.06.025

Nijland, R., and Burgess, J. G. (2010). Bacterial olfaction. *Biotechnol. J.* 5, 974–977. doi: 10.1002/biot.201000174

Oliver, A., Baquero, F., and Blazquez, J. (2002). The mismatch repair system (mutS, mutL and uvrD genes) in *Pseudomonas aeruginosa*: molecular characterization of naturally occurring mutants. *Mol. Microbiol.* 43, 1641–1650. doi: 10.1046/j.1365-2958.2002.02855.x

Palmer, R. J. Jr., Kazmerzak, K., Hansen, M. C., and Kolenbrander, P. E. (2001). Mutualism versus independence: strategies of mixed-species oral biofilms in vitro using saliva as the sole nutrient source. *Infect. Immun.* 69, 5794–5804. doi: 10.1128/IAI.69.9.5794-5804.2001

Pamp, S. J., Sternberg, C., and Tolker-Nielsen, T. (2009). Insight into the microbial multicellular lifestyle via flow-cell technology and confocal microscopy. *Cytometry A* 75, 90–103. doi: 10.1002/cyto.a.20685

Pande, S., Kaftan, F., Lang, S., Svatos, A., Germerodt, S., and Kost, C. (2016). Privatization of cooperative benefits stabilizes mutualistic cross-feeding interactions in spatially structured environments. *ISME J.* 10, 1413–1423. doi: 10.1038/ismej.2015.212

Pandin, C., Le Coq, D., Canette, A., Aymerich, S., and Briandet, R. (2017). Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? *Microb. Biotechnol.* 10, 719–734. doi: 10.1111/1751-7915.12693

Perchat, S., Talagas, A., Poncet, S., Lazar, N., Li de la Sierra-Gallay, I., Gohar, M., et al. (2016). How quorum sensing connects sporulation to necrotrophism in *Bacillus thuringiensis*. *PLoS Pathog.* 12:e1005779. doi: 10.1371/journal.ppat.1005779

Perez-Nunez, D., Briandet, R., David, B., Gautier, C., Renault, P., Hallet, B., et al. (2011). A new morphogenesis pathway in bacteria: unbalanced activity of cell wall synthesis machineries leads to coccus-to-rod transition and filamentation in ovococci. *Mol. Microbiol.* 79, 759–771. doi: 10.1111/j.1365-2958.2010.07483.x

Perrin, C., Briandet, R., Jubelin, G., Lejeune, P., Mandrand-Berthelot, M. A., Rodrigue, A., et al. (2009). Nickel promotes biofilm formation by *Escherichia coli* K-12 strains that produce curli. *Appl. Environ. Microbiol.* 75, 1723–1733. doi: 10.1128/AEM.02171-08

Peterson, B. W., He, Y., Ren, Y., Zerdoum, A., Libera, M. R., Sharma, P. K., et al. (2015). Viscoelasticity of biofilms and their recalcitrance to mechanical and chemical challenges. *FEMS Microbiol. Rev.* 39, 234–245. doi: 10.1093/femsre/fuu008

Piard, J. C., and Briandet, R. (2016). *Lactic Acid Bacteria Biofilms. Biotechnology of Lactic Acid Bacteria: Novel Applications*, 2nd Edn. Hoboken, NJ: Wiley-Blackwell, 341–361.

Pinna, D. (2014). Biofilms and lichens on stone monuments: do they damage or protect? *Front. Microbiol.* 5:133. doi: 10.3389/fmicb.2014.00133

Poltak, S. R., and Cooper, V. S. (2011). Ecological succession in long-term experimentally evolved biofilms produces synergistic communities. *ISME J.* 5, 369–378. doi: 10.1038/ismej.2010.136

Qayyum, S., Sharma, D., Bisht, D., and Khan, A. U. (2016). Protein translation machinery holds a key for transition of planktonic cells to biofilm state in *Enterococcus faecalis*: A proteomic approach. *Biochem. Biophys. Res. Commun.* 474, 652–659. doi: 10.1016/j.bbrc.2016.04.145

Qian, Z., Stoodley, P., and Pitt, W. G. (1996). Effect of low-intensity ultrasound upon biofilm structure from confocal scanning laser microscopy observation. *Biomaterials* 17, 1975–1980. doi: 10.1016/0142-9612(96)00022-1

Rendueles, O., and Ghigo, J. M. (2015). Mechanisms of competition in biofilm communities. *Microbiol. spectr.* 3:MB-0009-2014. doi: 10.1128/microbiolspec.MB-0009-2014

Rendueles, O., Beloin, C., Latour-Lambert, P., and Ghigo, J. M. (2014). A new biofilm-associated colicin with increased efficiency against biofilm bacteria. *ISME J.* 8, 1275–1288. doi: 10.1038/ismej.2013.238

Resch, A., Leicht, S., Saric, M., Pasztor, L., Jakob, A., Gotz, F., et al. (2006). Comparative proteome analysis of *Staphylococcus aureus* biofilm and planktonic cells and correlation with transcriptome profiling. *Proteomics* 6, 1867–1877. doi: 10.1002/pmic.200500531

Reuter, M., Mallett, A., Pearson, B. M., and van Vliet, A. H. (2010). Biofilm formation by *Campylobacter jejuni* is increased under aerobic conditions. *Appl. Environ. Microbiol.* 76, 2122–2128. doi: 10.1128/AEM.01878-09

Rhodes, C. J. (2016). The 2015 Paris climate change conference: COP21. *Sci. Prog.* 99, 97–104. doi: 10.3184/003685016X14528569315192

Rieu, A., Aoudia, N., Jego, G., Chluba, J., Yousfi, N., Briandet, R., et al. (2014). The biofilm mode of life boosts the anti-inflammatory properties of *Lactobacillus*. *Cell. Microbiol.* 16, 1836–1853. doi: 10.1111/cmi.12331

Romani, A. M., Borrego, C. M., Diaz-Villanueva, V., Freixa, A., Gich, F., and Ylla, I. (2014). Shifts in microbial community structure and function in light- and dark-grown biofilms driven by warming. *Environ. Microbiol.* 16, 2550–2567. doi: 10.1111/1462-2920.12428

Romero, D., Sanabria-Valentin, E., Vlamakis, H., and Kolter, R. (2013). Biofilm inhibitors that target amyloid proteins. *Chem. Biol.* 20, 102–110. doi: 10.1016/j.chembiol.2012.10.021

Romling, U., Galperin, M. Y., and Gomelsky, M. (2013). Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. *Microbiol. Mol. Biol. Rev.* 77, 1–52. doi: 10.1128/MMBR.00043-12

Sabater, S., Timoner, X., Borrego, C., and Acuña, V. (2016). Stream biofilm responses to flow intermittency: from cells to ecosystems. *Front. Environ. Sci.* 4:14. doi: 10.3389/fenvs.2016.00014

Safari, A., Habimana, O., Allen, A., and Casey, E. (2014). The significance of calcium ions on *Pseudomonas fluorescens* biofilms - a structural and mechanical study. *Biofouling* 30, 859–869. doi: 10.1080/08927014.2014.938648

Sauer, K. (2003). The genomics and proteomics of biofilm formation. *Genome Biol.* 4:219. doi: 10.1186/gb-2003-4-6-219

Sauer, K., Camper, A. K., Ehrlich, G. D., Costerton, J. W., and Davies, D. G. (2002). *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J. Bacteriol.* 184, 1140–1154. doi: 10.1128/jb.184.4.1140-1154.2002

Sauer, K., Cullen, M. C., Rickard, A. H., Zeef, L. A., Davies, D. G., and Gilbert, P. (2004). Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. *J. Bacteriol.* 186, 7312–7326. doi: 10.1128/JB.186.21.7312-7326.2004

Savage, V. J., Chopra, I., and O'Neill, A. J. (2013). *Staphylococcus aureus* biofilms promote horizontal transfer of antibiotic resistance. *Antimicrob. Agents Chemother.* 57, 1968–1970. doi: 10.1128/AAC.02008-12

Schilcher, K., Andreoni, F., Dengler Haunreiter, V., Seidl, K., Hasse, B., and Zinkernagel, A. S. (2016). Modulation of *Staphylococcus aureus* biofilm matrix by subinhibitory concentrations of clindamycin. *Antimicrob. Agents Chemother.* 60, 5957–5967. doi: 10.1128/AAC.00463-16

Schooling, S. R., and Beveridge, T. J. (2006). Membrane vesicles: an overlooked component of the matrices of biofilms. *J. Bacteriol.* 188, 5945–5957. doi: 10.1128/JB.00257-06

Schwartz, K., Ganesan, M., Payne, D. E., Solomon, M. J., and Boles, B. R. (2016). Extracellular DNA facilitates the formation of functional amyloids in *Staphylococcus aureus* biofilms. *Mol. Microbiol.* 99, 123–134. doi: 10.1111/mmi.13219

Schwechheimer, C., and Kuehn, M. J. (2015). Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. *Nat. Rev. Microbiol.* 13, 605–619. doi: 10.1038/nrmicro3525

Serra, D. O., Richter, A. M., Klauck, G., Mika, F., and Hengge, R. (2013). Microanatomy at cellular resolution and spatial order of physiological differentiation in a bacterial biofilm. *mBio* 4:e100103-13. doi: 10.1128/mBio.00103-13

Servick, K. (2016). DRUG DEVELOPMENT. Beleaguered phage therapy trial presses on. *Science* 352:1506. doi: 10.1126/science.352.6293.1506

Shafreen, R. M., Srinivasan, S., Manisankar, P., and Pandian, S. K. (2011). Biofilm formation by *Streptococcus pyogenes*: modulation of exopolysaccharide by fluoroquinolone derivatives. *J. Biosci. Bioeng.* 112, 345–350. doi: 10.1016/j.jbiosc.2011.06.013

Shank, E. A., Klepac-Ceraj, V., Collado-Torres, L., Powers, G. E., Losick, R., and Kolter, R. (2011). Interspecies interactions that result in *Bacillus subtilis* forming biofilms are mediated mainly by members of its own genus. *Proc. Natl. Acad. Sci. U.S.A.* 108, E1236–E1243. doi: 10.1073/pnas.1103630108

Shemesh, M., Kolter, R., and Losick, R. (2010). The biocide chlorine dioxide stimulates biofilm formation in *Bacillus subtilis* by activation of the histidine kinase KinC. *J. Bacteriol.* 192, 6352–6356. doi: 10.1128/JB.01025-10

Singer, G., Besemer, K., Schmitt-Kopplin, P., Hodl, I., and Battin, T. J. (2010). Physical heterogeneity increases biofilm resource use and its molecular diversity in stream mesocosms. *PLoS ONE* 5:e9988. doi: 10.1371/journal.pone.0009988

Skillman, L. C., Sutherland, I. W., and Jones, M. V. (1998). The role of exopolysaccharides in dual species biofilm development. *J. Appl. Microbiol.* 85 (Suppl. 1), 13S–18S. doi: 10.1111/j.1365-2672.1998.tb05278.x

Smith, W. P., Davit, Y., Osborne, J. M., Kim, W., Foster, K. R., and Pitt-Francis, J. M. (2017). Cell morphology drives spatial patterning in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 114, E280–E286. doi: 10.1073/pnas.1613007114

Solano, C., Echeverz, M., and Lasa, I. (2014). Biofilm dispersion and quorum sensing. *Curr. Opin. Microbiol.* 18, 96–104. doi: 10.1016/j.mib.2014.02.008

Soto, S. M. (2013). Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. *Virulence* 4, 223–229. doi: 10.4161/viru.23724

Srivastava, D., Harris, R. C., and Waters, C. M. (2011). Integration of cyclic di-GMP and quorum sensing in the control of vpsT and aphA in *Vibrio cholerae*. *J. Bacteriol.* 193, 6331–6341. doi: 10.1128/JB.05167-11

Srivastava, D., and Waters, C. M. (2012). A tangled web: regulatory connections between quorum sensing and cyclic Di-GMP. *J. Bacteriol.* 194, 4485–4493. doi: 10.1128/JB.00379-12

Stewart, E. J., Satorius, A. E., Younger, J. G., and Solomon, M. J. (2013). Role of environmental and antibiotic stress on *Staphylococcus epidermidis* biofilm microstructure. *Langmuir* 29, 7017–7024. doi: 10.1021/la401322k

Stewart, P. S., and Franklin, M. J. (2008). Physiological heterogeneity in biofilms. *Nat. Rev. Microbiol.* 6, 199–210. doi: 10.1038/nrmicro1838

Stipetic, L. H., Dalby, M. J., Davies, R. L., Morton, F. R., Ramage, G., and Burgess, K. E. (2016). A novel metabolomic approach used for the comparison of Staphylococcus aureus planktonic cells and biofilm samples. *Metabolomics* 12:75. doi: 10.1007/s11306-016-1002-0

Stoodley, P., Boyle, J. D., DeBeer, D., and Lappin-Scott, H. M. (1999). Evolving perspectives of biofilm structure. *Biofouling* 14, 75–90. doi: 10.1080/08927019909378398

Stoodley, P., Dodds, I., Boyle, J. D., and Lappin-Scott, H. M. (1998). Influence of hydrodynamics and nutrients on biofilm structure. *J. Appl. Microbiol.* 85(Suppl. 1), 19S–28S. doi: 10.1111/j.1365-2672.1998.tb05279.x

Stubbendieck, R. M., Vargas-Bautista, C., and Straight, P. D. (2016). Bacterial communities: interactions to scale. *Front. Microbiol.* 7:1234. doi: 10.3389/fmicb.2016.01234

Sutherland, I. W. (2001). The biofilm matrix–an immobilized but dynamic microbial environment. *Trends Microbiol.* 9, 222–227. doi: 10.1016/S0966-842X(01)02012-1

Toyofuku, M., Inaba, T., Kiyokawa, T., Obana, N., Yawata, Y., and Nomura, N. (2015). Environmental factors that shape biofilm formation. *Biosci. Biotechnol. Biochem.* 80, 7–12. doi: 10.1080/09168451.2015.1058701

Traverse, C. C., Mayo-Smith, L. M., Poltak, S. R., and Cooper, V. S. (2013). Tangled bank of experimentally evolved *Burkholderia* biofilms reflects selection during chronic infections. *Proc. Natl. Acad. Sci. U.S.A.* 110, E250–E259. doi: 10.1073/pnas.1207025110

Turonova, H., Briandet, R., Rodrigues, R., Hernould, M., Hayek, N., Stintzi, A., et al. (2015). Biofilm spatial organization by the emerging pathogen *Campylobacter jejuni*: comparison between NCTC 11168 and 81-176 strains under microaerobic and oxygen-enriched conditions. *Front. Microbiol.* 6:709. doi: 10.3389/fmicb.2015.00709

Ueda, A., and Wood, T. K. (2009). Connecting quorum sensing, c-di-GMP, pel polysaccharide, and biofilm formation in *Pseudomonas aeruginosa* through tyrosine phosphatase TpbA (PA3885). *PLoS Pathog.* 5:e1000483. doi: 10.1371/journal.ppat.1000483

Valentini, M., and Filloux, A. (2016). Biofilms and cyclic di-GMP (c-di-GMP) signaling: lessons from *Pseudomonas aeruginosa* and other bacteria. *J. Biol. Chem.* 291, 12547–12555. doi: 10.1074/jbc.R115.711507

Valle, J., Da Re, S., Henry, N., Fontaine, T., Balestrino, D., Latour-Lambert, P., et al. (2006). Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12558–12563. doi: 10.1073/pnas.0605399103

van Hoek, M. L. (2013). Biofilms: an advancement in our understanding of *Francisella* species. *Virulence* 4, 833–846. doi: 10.4161/viru.27023

Van Meervenne, E., De Weirdt, R., Van Coillie, E., Devlieghere, F., Herman, L., and Boon, N. (2014). Biofilm models for the food industry: hot spots for plasmid transfer? *Pathog. Dis.* 70, 332–338. doi: 10.1111/2049-632X.12134

Vilain, S., and Brozel, V. S. (2006). Multivariate approach to comparing whole-cell proteomes of *Bacillus cereus* indicates a biofilm-specific proteome. *J. Proteome Res.* 5, 1924–1930. doi: 10.1021/pr050402b

Vuotto, C., Longo, F., and Donelli, G. (2014). Probiotics to counteract biofilm-associated infections: promising and conflicting data. *Int. J. Oral Sci.* 6, 189–194. doi: 10.1038/ijos.2014.52

Waite, R. D., Paccanaro, A., Papakonstantinopoulou, A., Hurst, J. M., Saqi, M., Littler, E., et al. (2006). Clustering of *Pseudomonas aeruginosa* transcriptomes from planktonic cultures, developing and mature biofilms reveals distinct expression profiles. *BMC Genomics* 7:162. doi: 10.1186/1471-2164-7-162

Wang, W., Chanda, W., and Zhong, M. (2015). The relationship between biofilm and outer membrane vesicles: a novel therapy overview. *FEMS Microbiol. Lett.* 362:fnv117. doi: 10.1093/femsle/fnv117

Watnick, P., and Kolter, R. (2000). Biofilm, city of microbes. *J. Bacteriol.* 182, 2675–2679. doi: 10.1128/JB.182.10.2675-2679.2000

Webb, J. S., Thompson, L. S., James, S., Charlton, T., Tolker-Nielsen, T., Koch, B., et al. (2003). Cell death in *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.* 185, 4585–4592. doi: 10.1128/JB.185.15.4585-4592.2003

Werner, E., Roe, F., Bugnicourt, A., Franklin, M. J., Heydorn, A., Molin, S., et al. (2004). Stratified growth in *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.* 70, 6188–6196. doi: 10.1128/AEM.70.10.6188-6196.2004

Whiteley, M., Bangera, M. G., Bumgarner, R. E., Parsek, M. R., Teitzel, G. M., Lory, S., et al. (2001). Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature* 413, 860–864. doi: 10.1038/35101627

Widder, S., Allen, R. J., Pfeiffer, T., Curtis, T. P., Wiuf, C., Sloan, W. T., et al. (2016). Challenges in microbial ecology: building predictive understanding of community function and dynamics. *ISME J.* 10, 2557–2568. doi: 10.1038/ismej.2016.45

Wong, H. S., Maker, G. L., Trengove, R. D., and O'Handley, R. M. (2015). Gas chromatography-mass spectrometry-based metabolite profiling of *Salmonella enterica* serovar Typhimurium differentiates between biofilm and planktonic phenotypes. *Appl. Environ. Microbiol.* 81, 2660–2666. doi: 10.1128/AEM.03658-14

Wright, A., Hawkins, C. H., Anggard, E. E., and Harper, D. R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clin. Otolaryngol.* 34, 349–357. doi: 10.1111/j.1749-4486.2009.01973.x

Xu, J., Li, M., He, Q., Sun, X., Zhou, X., Su, Z., et al. (2017). Effect of flow rate on growth and oxygen consumption of biofilm in gravity sewer. *Environ. Sci. Pollut. Res. Int.* 24, 427–435. doi: 10.1007/s11356-016-7710-1

Yan, J., Nadell, C. D., and Bassler, B. L. (2017). Environmental fluctuation governs selection for plasticity in biofilm production. *ISME J.* 11, 1569–1577. doi: 10.1038/ismej.2017.33

Yan, J., Sharo, A. G., Stone, H. A., Wingreen, N. S., and Bassler, B. L. (2016). *Vibrio cholerae* biofilm growth program and architecture revealed by single-cell live

imaging. *Proc. Natl. Acad. Sci. U.S.A.* 113, E5337–E5343. doi: 10.1073/pnas.1611494113

Yang, Y., Sreenivasan, P. K., Subramanyam, R., and Cummins, D. (2006). Multiparameter assessments to determine the effects of sugars and antimicrobials on a polymicrobial oral biofilm. *Appl. Environ. Microbiol.* 72, 6734–6742. doi: 10.1128/AEM.01013-06

Ye, Y., Jiao, R., Gao, J., Li, H., Ling, N., Wu, Q., et al. (2016). Proteins involved in responses to biofilm and planktonic modes in *Cronobacter sakazakii*. *LWT Food Sci. Technol.* 65, 1093–1099. doi: 10.1016/j.lwt.2015.09.039

Yonezawa, H., Osaki, T., Woo, T., Kurata, S., Zaman, C., Hojo, F., et al. (2011). Analysis of outer membrane vesicle protein involved in biofilm formation of *Helicobacter pylori*. *Anaerobe* 17, 388–390. doi: 10.1016/j.anaerobe.2011.03.020

Zeriouh, H., de Vicente, A., Perez-Garcia, A., and Romero, D. (2014). Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. *Environ. Microbiol.* 16, 2196–2211. doi: 10.1111/1462-2920.12271

Zhang, L., and Mah, T. F. (2008). Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. *J. Bacteriol.* 190, 4447–4452. doi: 10.1128/JB.01655-07

Zhou, H., Luo, C., Fang, X., Xiang, Y., Wang, X., Zhang, R., et al. (2016). Loss of GltB inhibits biofilm formation and biocontrol efficiency of *Bacillus subtilis* Bs916 by altering the production of gamma-polyglutamate and three lipopeptides. *PLoS ONE* 11:e0156247. doi: 10.1371/journal.pone.0156247

Ziemba, C., Shabtai, Y., Piatkovsky, M., and Herzberg, M. (2016). Cellulose effects on morphology and elasticity of *Vibrio fischeri* biofilms. *npj Biofilms Microbiomes* 2:1. doi: 10.1038/s41522-016-0001-2

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.2 Article 2: "Should the biofilm mode of life be taken into consideration for microbial biocontrol agents"

Caroline Pandin[1], Dominique Le Coq[1,2], Alexis Canette[1], Stéphane Aymerich[1], Romain Briandet[1]

[1] Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[2] Micalis Institute, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78350 Jouy-en-Josas, France.

Microbial Biotechnology, 2017

(http://dx.doi.org/10.1111/1751-7915.12693)

microbial biotechnology
Open Access

Minireview

Should the biofilm mode of life be taken into consideration for microbial biocontrol agents?

Caroline Pandin,[1] Dominique Le Coq,[1,2] Alexis Canette,[1] Stéphane Aymerich[1] and Romain Briandet[1,*]
[1]*Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.*
[2]*Micalis Institute, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78350 Jouy-en-Josas, France.*

Summary

Almost one-third of crop yields are lost every year due to microbial alterations and diseases. The main control strategy to limit these losses is the use of an array of chemicals active against spoilage and unwanted pathogenic microorganisms. Their massive use has led to extensive environmental pollution, human poisoning and a variety of diseases. An emerging alternative to this chemical approach is the use of microbial biocontrol agents. Biopesticides have been used with success in several fields, but a better understanding of their mode of action is necessary to better control their activity and increase their use. Very few studies have considered that biofilms are the preferred mode of life of microorganisms in the target agricultural biotopes. Increasing evidence shows that the spatial organization of microbial communities on crop surfaces may drive important bioprotection mechanisms. The aim of this review is to summarize the evidence of biofilm formation by biocontrol agents on crops and discuss how this surface-associated mode of life may influence their biology and interactions with other microorganisms and the host and, finally, their overall beneficial activity.

Introduction

Approximately 30% of crop yields are lost every year worldwide, mostly due to diseases caused by pests, weeds or pathogenic microorganisms (Teng and Krupa, 1980; Teng, 1987; Oerke, 1999, 2006; Savary *et al.*, 2012). The microbiological control of agricultural products along the food chain is still mainly ensured by the extensive use of chemical pesticides, preservatives and synthetic drugs (Horrigan *et al.*, 2002). Environmental pollution and associated human diseases caused by this excessive use of chemicals during last century has led many agencies and governments worldwide to support an alternative route, where agriculture can be productive and economically viable, while still addressing societal and environmental concerns (Anonymous, 1999; Hazell and Wood, 2008; Aktar *et al.*, 2009). Biological protection strategies are used and encouraged from farm to forks to prevent pathogen contaminations and livestock or crop diseases (Pal and McSpadden Gardener, 2006; Sundh and Melin, 2010; Jordan *et al.*, 2014). Biological control, or 'biocontrol', consists in the removal of the harmful activity of one organism via one or more organisms or natural products extracted from microorganisms, plants, animals or minerals (Pal and McSpadden Gardener, 2006).

The relationship between survival, persistence and virulence of pathogenic microorganisms with their biofilm mode of life have been clearly established since the early 1980s (Costerton *et al.*, 1978; Lam *et al.*, 1980). According to the National Institute of Health, 80% of human infections involves microbial biofilms (NIH, 2002). Biofilm-associated infections have also been reported in agricultural settings, e.g., in crops and animal diseases (Davey and O'toole, 2000; Prigent-Combaret *et al.*, 2012; Li *et al.*, 2015). Indeed, the sessile mode is the preferential lifestyle of microorganisms, regardless of their biotope (Davey and O'toole, 2000; Morris and Monier, 2003). A biofilm can be described as a spatially structured community of microorganisms, generally embedded in an extracellular matrix, and adhering to a living or inert surface (Costerton *et al.*, 1999; O'Toole *et al.*, 2000). Biofilm formation is generally favoured in harsh environmental conditions, such as low nutritive or toxic media (Rendueles and Ghigo, 2015) and most bacteria can form biofilms in various environments (Morris and Monier, 2003; Aparna and Yadav, 2008). *Staphylococcus aureus* and *Pseudomonas aeruginosa* are two opportunistic pathogenic

Received 15 July, 2016; revised 19 January, 2017; accepted 20 January, 2017. *For correspondence. E-mail romain.briandet@inra.fr; Tel. +33 1 74 07 16 98.
Microbial Biotechnology (2017) **00**(00), 000–000
doi:10.1111/1751-7915.12693

bacteria that cause a diverse set of diseases and are the most highly used model bacteria for biofilm studies. They can colonize the human nasopharynx and form biofilms when specific environmental conditions are met, causing invasive diseases, such as chronic pneumonia. These infections are difficult to treat because of the persistence of biofilms and their high resistance to antimicrobials (Blanchette and Orihuela, 2012; Ding *et al.*, 2016a). Bacteria can colonize and form biofilms on stems, leaves and the rhizosphere of plants, as well as soil particles, mushrooms or organic compost (Figs 1A and 2) (Ramey *et al.*, 2004; Prigent-Combaret *et al.*, 2012). For example, *Dickeya dadantii*, the causal agent of soft rot disease in a wide range of plant species, can colonize and form biofilms on chicory leaves, causing disease due to the production of degradative enzymes (Prigent-Combaret *et al.*, 2012; Pandin *et al.*, 2016).

Although less explored, the formation of biofilms by moulds, yeast and algae, alone or in combination, in a variety of biotopes has also been reported (Morris and Monier, 2003; Aparna and Yadav, 2008; Zarnowski *et al.*, 2014; He *et al.*, 2016; Rajendran and Hu, 2016; Sheppard and Howell, 2016). *Aspergillus fumigatus,* a human pathogen, is a filamentous fungus that can form structured biofilms. The cohesive cement of the fungal biostructure is a polymeric extracellular matrix that protects the hyphae from the host immune system, similar to bacterial biofilms (Breitenbach *et al.*, 2016; Mitchell *et al.*, 2016; Sheppard and Howell, 2016; Shirazi *et al.*, 2016). *Fusarium oxysporum* f. sp. *cucumerinum*, the pathogen responsible for cucumber Fusarium wilt, can also grow inter- and intracellularly, allowing the rapid colonization of the plant and biofilm formation (Li *et al.*, 2015). Until recently, efforts in biofilm research have

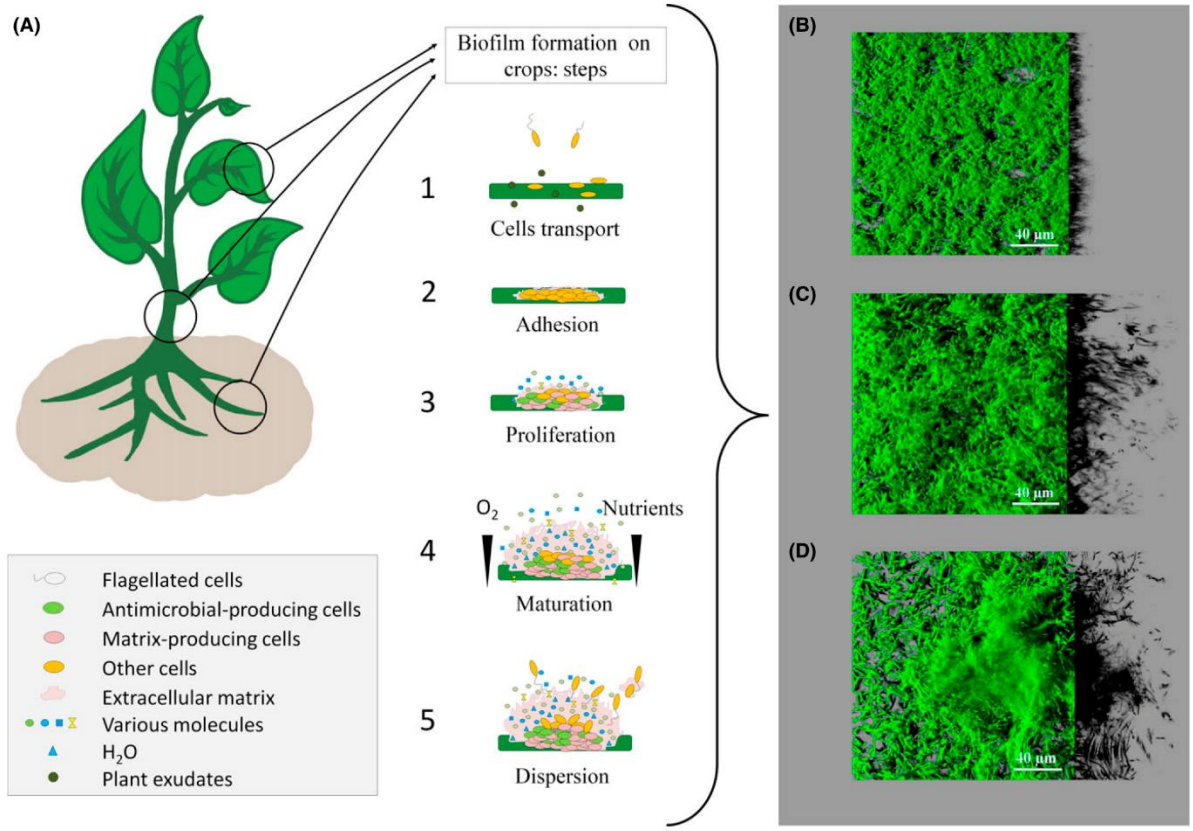

Fig. 1. Biofilm formation on crops and *in vitro*: (A): On crops: The first step involves deposition on the substratum (1) followed by adhesion (2) to the support through cell wall decorations and extracellular appendages. Once attached, a proliferation phase (3) and the diversification of cell types initiate the spatial organization of the biostructure, leading to biofilm maturation (4). Biofilm ageing or environmental conditions unfavourable for the maintenance of the biofilm results in regulated dispersion of the biofilm (5), disseminating free cells and cell clusters that will start a new biofilm cycle on a new surface.
B–D. *In vitro*: Structural diversity of three biocontrol agents as observed *in vitro* (24 h of axenic culture in microplates at 25°C) by confocal laser scanning microscopy (Leica SP8); (B) *Bacillus amyloliquefaciens* FZB42 expressing a green fluorescent protein (GFP), forming flat undifferentiated architecture, (C) *Bacillus amyloliquefaciens* SQR9 expressing a GFP and (D) *Bacillus subtilis* QST 713 (labelled in green with syto 9, Invitrogen, France) forming differentiated 3D biostructures.

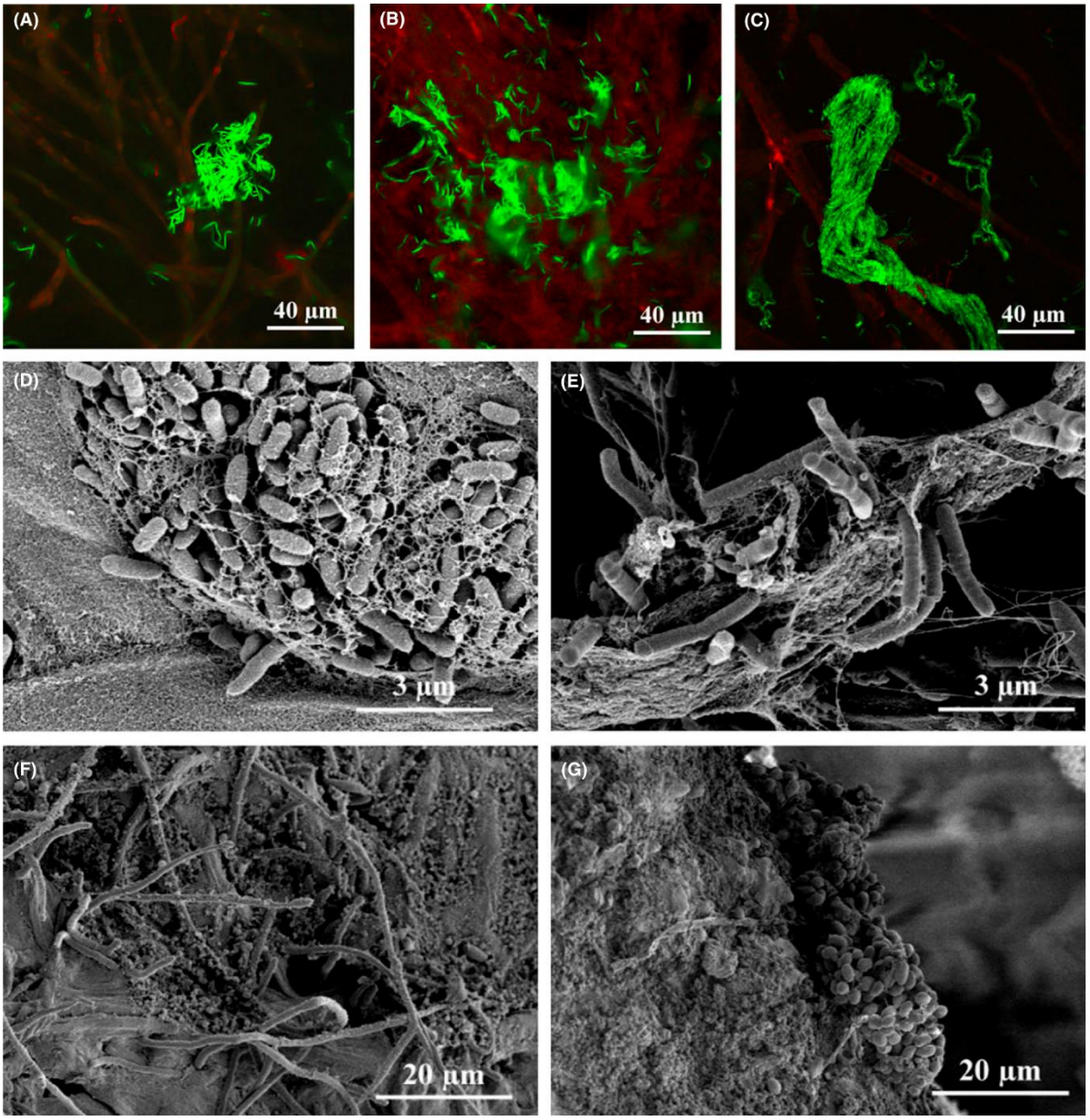

Fig. 2. Microbial biofilms on the carpophore and culture compost of *Agaricus bisporus*.
A–C. Confocal laser scanning microscopy of *Agaricus bisporus* carpophore (red autofluorescent hyphae), harbouring *Bacillus amyloliquefaciens* FZB42 expressing GFP and forming (A) clusters, (B) biofilm features and (C) bundles. *Agaricus bisporus* carpophores were immersed under axenic conditions in TSB (Tryptone Soy Broth, Sigma-Aldrich, France) inoculated with *Bacillus amyloliquefaciens* FZB42 (GFP tagged) and incubated for 48 h at 17°C. Observations were performed using a Leica SP8 (Leica Microsystems, Danaher, Germany).
D–G. Scanning electron microscopy of natural biofilms formed on *Agaricus bisporus* carpophore and compost protected with *Bacillus subtilis* QST 713, a biocontrol agent used at the French Mushroom Centre (Distré, France). Samples were fixed in 0.10 M cacodylate buffer containing 2.5% (v/v) glutaraldehyde (pH 7.4) and post-fixed in 1% osmium tetroxide. Samples were then dehydrated with increasing concentrations of ethanol at room temperature (50–100%). After drying, samples were mounted on grids, sputter-coated in argon plasma with platinum (Polaron SC7640, Elexience, France) and observed using a FE-SEM S4500 (Hitachi, Japan). (D) *Pseudomonas*-like bacteria with extracellular material, (E) *Bacillus*-like bacteria, (F) fungi hyphae with extracellular material, (G) bacterial microcolony.

focused mainly on the medical field and essentially towards their eradication. With the emergence of biocontrol in agriculture, many microbiological products have been developed and are used in fields (Borriss, 2015). The main way of action of most of these commercial products is the antagonistic effect of antimicrobial

molecules secreted by the biocontrol agent (Chowdhury *et al.*, 2015; Mora *et al.*, 2015). However, recent research in this field has made it possible to consider other major biological processes, including biofilm formation of biocontrol agents in crops (Bais *et al.*, 2004; Bogino *et al.*, 2013; De la Fuente *et al.*, 2013).

The formation of biofilms by microbial biocontrol agents

Evidence of biofilm formation on crops by biocontrol agents

There is ongoing research to identify new biocontrol agents from environmental isolates and numerous biocontrol products have been developed and put on the agricultural market, mostly in Europe and North America (Borriss, 2015). Various products are in use and are effective on a wide range of plants. These include biofungicides, bactericides and biofertilizers based on *Bacillus subtilis* QST 713 or *Bacillus amyloliquefaciens* FZB42 (Borriss, 2015). These biocontrol products have an antagonistic effect towards unwanted microbes due to their secretion of antimicrobials, such as surfactin, fengycin or iturin (Ongena *et al.*, 2005; Ongena and Jacques, 2008; Cawoy *et al.*, 2014, 2015; Saravanakumar *et al.*, 2016). However, their precise mechanisms of action in fields are still unknown. Few studies have focused on the determinants of effective bioprotection. The surface colonization step and biofilm formation by biocontrol agents are highlighted in the publications cited in Table 1. These reports demonstrate that many biocontrol agents can form biofilms on crops and in the rhizosphere. It has also been shown that biofilm formation by biopesticides can be stimulated by plant root exudates (Espinosa-Urgel *et al.*, 2002; Timmusk *et al.*, 2005; Haggag and Timmusk, 2008; Khezri *et al.*, 2011; Chen *et al.*, 2013; Sang and Kim, 2014; Zhang *et al.*, 2015), or by exposure of the microorganisms to antimicrobial products or stress (Bais *et al.*, 2004; Selin *et al.*, 2010; Fan *et al.*, 2011; Xu *et al.*, 2014; Chi *et al.*, 2015; Wu *et al.*, 2015; Zhou *et al.*, 2016), but only a few studies have focused on biocontrol mechanisms that may be related to the properties of the mature biofilm itself, rather than the secretion of antimicrobials. *Bacillus* are ubiquitous spore forming bacteria predominantly found in soil. They are frequently used as biocontrol agents because they can sporulate and be stored for long periods (Branda *et al.*, 2004; Borriss, 2015). *Bacillus amyloliquefaciens* FZB42 forms biofilms with little spatial organization *in vitro* (Fig. 1B), but exhibits a strong swarming capacity allowing a rapid surface colonization. For example, this strain can form biofilms on the fruiting body of *Agaricus bisporus* by forming bacterial clusters surrounded by extracellular matrix in contact with the mycelium of the carpophore (Fig. 2A and B), as well as cell bundles (Fig. 2C). Fan *et al.* (2011) reported the induction of biofilm formation of *B. amyloliquefaciens* FZB42 by root exudates of maize and surfactin. Similarly, surfactin triggers biofilm formation by *B. subtilis* UMAF6614 on the melon phylloplane (Zeriouh *et al.*, 2014). Root exudate of cucumber also drives the chemotaxis of *Bacillus amyloliquefaciens* SQR9 and induces the production of bacillomycin D that triggers biofilm formation in the rhizosphere (Xu *et al.*, 2014). Similarly, stem lesions of rice induce the production of GltB, leading to the production of bacillomycin L and surfactin, both involved in the biofilm formation of *B. subtilis* Bs916 (Zhou *et al.*, 2016). Other biocontrol agents, such as endophytes, can also form biofilms. For example, some bacteria of the genus *Paenibacillus* form biofilms in wheat seeds and protect them from the invasion of *Fusarium graminearum* (Díaz Herrera *et al.*, 2016).

Another family of biocontrol agents consists of the Gram-negative *Pseudomonas*, ubiquitous bacteria found in many plant rhizospheres (Table 1) (Espinosa-Urgel *et al.*, 2002; Steddom *et al.*, 2002; Matilla *et al.*, 2010; Selin *et al.*, 2010). Biofilm formation by *Pseudomonas putida* 06909 on citrus roots is induced by exudates of the phytopathogen *Phytophthora parasitica.* The bacteria colonize the mycelium of the fungi by feeding on its exudates and then form a protective biofilm on the citrus roots, which prevents new growth of the pathogen (Steddom *et al.*, 2002; Ahn *et al.*, 2007).

Living in a biofilm profoundly alters microbial properties relative to the planktonic mode of life (Whiteley *et al.*, 2001; Shemesh *et al.*, 2007; Vlamakis *et al.*, 2008, 2013; Bridier *et al.*, 2011b). Ongoing research is currently deciphering the molecular mechanisms involved in biofilm formation and their repercussions on biocontrol efficacy.

Molecular mechanisms involved in biofilm formation of biocontrol agents

Until recently, few studies in the biocontrol field have considered that the preferred lifestyle of microorganisms in the environment is the biofilm mode of life. The main features associated with biofilm formation are a diversification of cell types and increased tolerance to the fluctuation of environmental factors, boosting microbial persistence in the environment (Vlamakis *et al.*, 2008, 2013; Flemming *et al.*, 2016). Bacteria and fungi can form biofilms on crops (as illustrated by the cultivated mushroom microbiota in Fig. 2), and in both cases, biofilm formation is composed of five major steps described in Fig. 1A (Costerton *et al.*, 1999; O'Toole *et al.*, 2000; Bianciotto *et al.*, 2001; Davies, 2003; Triveni *et al.*, 2012; Vlamakis *et al.*, 2013; Pu *et al.*, 2014; Haagensen

Table 1. Biocontrol agent reported to form biofilms and the described associated biocontrol mechanisms.

Biocontrol strain	Host/Location	Biofilm induction	Biocontrol mechanism	References
Bacillus atrophaeus 176s	Lettuce, sugar beet, tomato	Surfactin triggers biofilm formation	Induced systemic resistance (ISR) antimicrobial-producing biofilm (fengycin, surfactin)	(Aleti *et al*., 2016)
Bacillus subtilis	Wheat seeds	Root exudates, death or lysis of cortex cells	Biofilm formation, antimicrobial, volatile compounds decrease mycelial growth	(Khezri *et al*., 2011)
Bacillus subtilis 3610	Tomato roots	Root exudates induce matrix	Antimicrobial-producing biofilm (surfactin)	(Chen *et al*., 2013)
Bacillus subtilis 6051	*Arabidopsis thaliana*	Surfactin triggers biofilm formation	Antimicrobial-producing biofilm (surfactin)	(Bais *et al*., 2004)
Bacillus subtilis Bs916	Rice stem	Stem lesions induce GltB production triggering bacillomycin L and, surfactin production involved in biofilm formation	Antimicrobial-producing biofilm (fengycin)	(Zhou *et al*., 2016)
Bacillus subtilis UMAF6614	Melon phylloplane	Surfactin triggers biofilm formation	Antimicrobial-producing biofilm (bacillomycin, fengycin)	(Zeriouh *et al*., 2014)
Bacillus amyloliquefaciens SQR9	Cucumber roots	Root exudates induce chemotaxis and enhance bacillomycin D production	Antimicrobial-producing biofilm (bacillomycin)	(Xu *et al*., 2014)
Bacillus amyloliquefaciens SQR9	Maize roots	Root exudates induce the expression of genes related to extracellular matrix production	Promote plant growth	(Zhang *et al*., 2015)
Bacillus amyloliquefaciens SQY 162	Tobacco roots	Pectin enhances surfactin production, increasing biofilm biomass	May trigger induced systemic resistance (ISR) antimicrobial-producing biofilm (surfactin)	(Wu *et al*., 2015)
Bacillus amyloliquefaciens FZB42	Maize roots	Root exudates and surfactin trigger biofilm formation	Likely not linked with the production of antibiotic or biofilm formation	(Fan *et al*., 2011)
Paenibacillus polymyxa	*Arabidopsis thaliana*	Root exudates induce matrix synthesis	Niche exclusion and mechanical protection	(Timmusk *et al*., 2005)
Paenibacillus polymyxa A26	Wheat seeds	Not mentioned	Niche exclusion of pathogens	(Abd El Daim *et al*., 2015)
Paenibacillus polymyxa B5	*Arabidopsis thaliana*	Root exudates	Niche exclusion of pathogens	(Haggag and Timmusk, 2008)
Pseudomonas corrugata CCR04 and CCR80	Pepper roots	Root exudates	Competitive colonization, such as swimming and swarming activities, biofilm formation, antimicrobial activity	(Sang and Kim, 2014)
Pseudomonas chlororaphis PA23	Canola roots	Phenazine enhances biofilm formation	Antimicrobial-producing biofilm (pyrrolnitrin)	(Selin *et al*., 2010)
Pseudomonas putida 06909	Citrus roots	*Phytophthora* exudates as attractants and growth substrates for bacteria	Biofilm formation and mycelial colonization of the pathogen *Phytophtora*	(Steddom *et al*., 2002; Ahn *et al*., 2007)
Pseudomonas putida KT2440	Corn roots *Arabidopsis thaliana*	Root exudates	Promote plant growth and induced systemic resistance (ISR)	(Espinosa-Urgel *et al*., 2002; Matilla *et al*., 2010)
Pichia kudriavzevii	Pear fruit	Oxidative stress	Greater activation of the antioxidant system in the biofilm form	(Chi *et al*., 2015)
Kloeckera apiculate	Citrus fruit	Phenylethanol promotes filamentous adhesion and biofilm formation	Niche exclusion and mechanical protection	(Pu *et al*., 2014)

et al., 2015; Li *et al*., 2015; Gulati and Nobile, 2016; Sheppard and Howell, 2016). *Bacillus subtilis* is the most highly documented bacterial model currently used to study the regulatory molecular mechanisms that govern biofilm formation. One specificity of the biofilm mode of life is the diversification of cell types. The presence of several bacterial subpopulations within the biofilm of *B. subtilis* has been clearly demonstrated, suggesting the spatiotemporal regulation of gene expression within such 3D structures (Vlamakis *et al*., 2008, 2013). Matrix-producing cells, surfactin-producing cells, flagellated motile cells and sporulated cells coexist in the same community (Fig. 1A) and are spatially and temporally organized, differentially expressing specific sets of genes (Vlamakis *et al*., 2008; van Gestel *et al*., 2015; Mielich-Süss and Lopez, 2015; Wang *et al*., 2016). Indeed, the combination of surfactin- and matrix-producing cells enables the organization of cells into bundles (Fig. 2C). These interfacial microbial cables allow bacteria to visit surrounding spaces to increase the biofilm surface area for nutrient and oxygen intake (van Gestel *et al*., 2015). Several genes involved in the phenotypic heterogeneity have been identified and extensively analysed in this species. For example, *hag*, encoding a flagellar protein

and expressed by a subpopulation of motile cells; *tasA, eps, blsA* expressed by matrix-producing cells; *sfrA*, involved in the production of surfactin lipopeptide; *sigF*, involved in cell sporulation; *swr*, involved in swarming motility; and the *com* genes, involved in genetic competence (Kearns *et al*., 2004; Verhamme *et al*., 2007; López and Kolter, 2010; Vlamakis *et al*., 2013; van Gestel *et al*., 2015; Mielich-Süss and Lopez, 2015). All these genes are directly or indirectly regulated by various regulators (e.g. Spo0A, DegU, ComA, SinI, SinR, AbrB), which can thus play a role in the regulation of plant bioprotection by *B. subtilis* (López and Kolter, 2010; Vlamakis *et al*., 2013; Cairns *et al*., 2014; Mielich-Süss and Lopez, 2015; Romero *et al*., 2016). Indeed, a mutation in a gene coding for a positive regulator (e.g. SinI) will decrease plant colonization and protection by diminishing attachment of cells to the roots, while mutations in a gene coding for a repressor (e.g. SinR, AbrB) will increase plant protection by an increased numbers of root-attached cells and the formation of hyper-robust biofilms (Chen *et al*., 2013).

Major components of biofilm structure that ensure its cohesion are the extracellular polymeric substances (EPS) that are mostly composed of water and extracellular biopolymers (polysaccharides, proteins, DNA, lipids) (Flemming and Wingender, 2010). Many microbial EPS have a backbone composed of various biomolecules forming gels with various cohesive and viscoelastic properties. Trapping a high amount of water is important for microbial survival against desiccation on plant surfaces (Abdian and Zorreguieta, 2016). This organic slime also protects their inhabitants from the action of environmental pollutants and toxic compounds (Sutherland, 2001; Sheppard and Howell, 2016). Another important component of the biofilm structure are amyloid fibres formed by the protein TasA. These filaments bind cells together, leading to formation of complex structures in biofilms that can hold and concentrate molecules (e.g. quorum sensing signalling molecules), and may also form aggregates to defend cells within the biofilm (de Jong *et al*., 2009; Romero *et al*., 2010; Flemming *et al*., 2016).

Several studies have recently highlighted various physiological behaviours of *Bacillus* within biofilm communities, demonstrating the high level of complexity of their interactions (Mitri *et al*., 2011; Houry *et al*., 2012; Liu *et al*., 2015; Prindle *et al*., 2015; Flemming *et al*., 2016). Prindle *et al.* (2015) described a new function for ion channels in biofilms in which they conduct electrical signals *via* spatial propagation of potassium waves which depolarize adjoining cells and coordinate the state of the exterior and interior cells of the biofilm. In addition, Liu *et al.* (2015) discovered a 'collective oscillation' phenomenon involved in toxic chemical tolerance, based on metabolic codependency between exterior and interior cells of the biofilm, and consisting of cyclic pauses during biofilm growth which increase the availability of nutrients in the deepest layers. Houry *et al.* (2012) also demonstrated that motile bacilli, expressing a bactericide, can kill a heterologous biofilm population and then occupy the newly created space (Houry *et al*., 2012). Altogether, these cellular traits show the complexity of living associated with a surface in a spatially organized microbial community. They also give an overview of the protection that biofilms can provide to their inhabitants on plant surfaces. Those basic insights into biofilm development and interaction might pave our way towards various applications in the field of crop protection.

Biofilm-specific properties that should be considered in biocontrol mechanisms

Only a few published studies have considered the possibility of interspecies and microbial–host interactions in spatially organized plurimicrobial biofilms involved in agricultural biocontrol (De la Fuente *et al*., 2013; Triveni *et al*., 2015) (Table 1). The biofilm-associated properties to be considered can be divided into five classes (Fig. 3): (i) antagonism by niche exclusion orchestrated by spatial and nutritive competition (Timmusk *et al*., 2005; Haggag and Timmusk, 2008; Pu *et al*., 2014; Abd El Daim *et al*., 2015), (ii) microbial communication, e.g. cooperation/interference (Hogan *et al*., 2004; Audrain *et al*., 2015; Chen *et al*., 2015), (iii) production of antimicrobials by biofilm cells (Bais *et al*., 2004; Selin *et al*., 2010; Chen *et al*., 2013; Sang and Kim, 2014; Xu *et al*., 2014; Zeriouh *et al*., 2014; Wu *et al*., 2015; Zhou *et al*., 2016), (iv) stress tolerance (Timmusk *et al*., 2005; Harriott and Noverr, 2009; Pu *et al*., 2014) and (v) direct effects on plant physiology, e.g. the induction of plant defences (Wu *et al*., 2015) and/or stimulation of plant growth (Espinosa-Urgel *et al*., 2002; Zhang *et al*., 2015). This new vision could significantly change our understanding of the interactions involved in biocontrol by considering them in terms of spatial/nutritive competition (Habimana *et al*., 2011), tolerance/resistance (Bridier *et al*., 2011a) or their physiology, as microorganisms in a biofilm differ greatly from their planktonic homologues (Stewart and Franklin, 2008). These local processes are described in the following sections, using illustrative examples from other fields, if they have not been explored yet in the microbial biocontrol area.

Spatial and nutritive competition

The spatial organization of biocontrol agent biofilms on crop surfaces varies depending on their genetic potential and the environmental conditions. For example, the

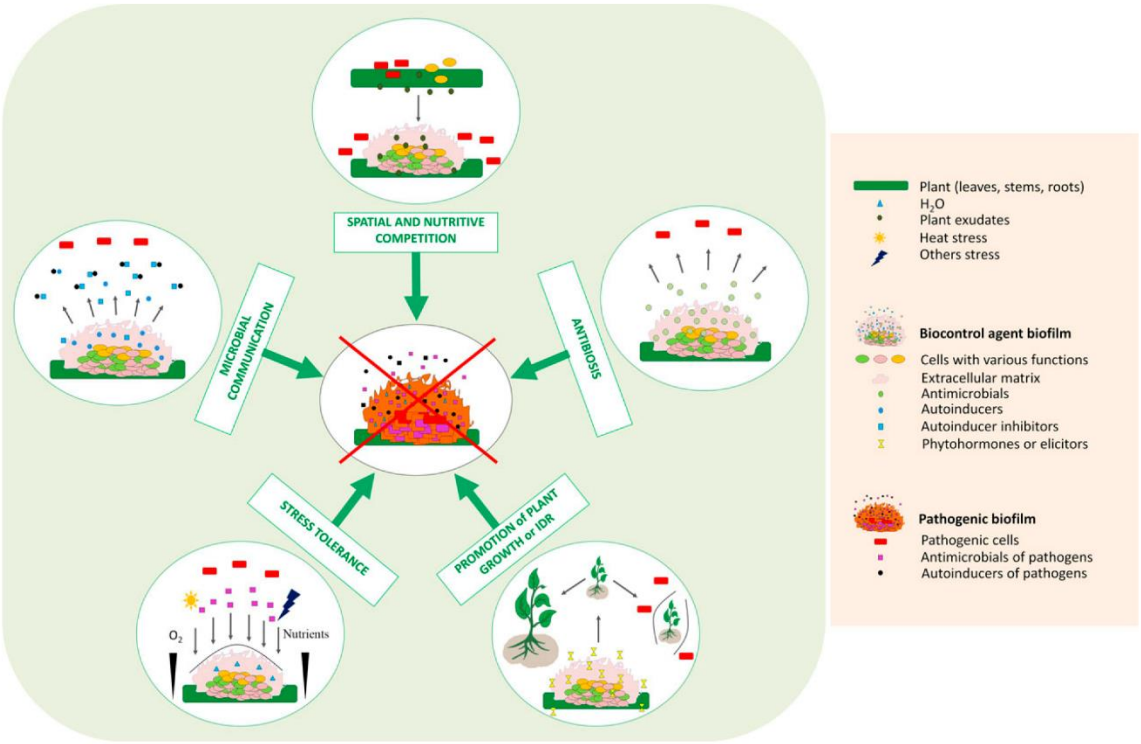

Fig. 3. Proposed mechanisms of plant interactions with biocontrol agents and pathogenic strains. (IDR: induced disease resistance).

biofilms of *Bacillus* biocontrol agents display wide architectural diversity between strains. *In vitro,* biofilms of *B. amyloliquefaciens* SQR9 and *B. subtilis* QST 713 exhibit the classical thick and highly organized 3D structure of bacilli (Fig. 1C and D). In contrast, *B. amyloliquefaciens* FZB42 forms only thin structures of a few cell layers (Fig. 1B). However, this strain outcompetes the other two due to its swarming activity, leading to rapid coverage of the entire surface. This ability to rapidly colonize a niche (Fig. 3) has been described previously as a potential biocontrol mechanism and could be called upon for the strain *B. amyloliquefaciens* FZB42 (Timmusk *et al.*, 2005; Haggag and Timmusk, 2008; Fan *et al.*, 2011; Abd El Daim *et al.*, 2015). For *Paenibacillus polymyxa,* root exudates of plants induce invasive root colonization and biofilm formation that invades sites that could be potentially occupied by pathogens, thus preventing them from settling onto the surface by forming a protective biofilm (Timmusk *et al.*, 2005; Haggag and Timmusk, 2008; Abd El Daim *et al.*, 2015). In an organized, 3D community, nutrients may be consumed faster than they can diffuse throughout the matrix (Breugelmans *et al.*, 2008; Stewart and Franklin, 2008). Growth and survival in such a dense community is frequently associated with spatial competition. Habimana *et al.* (2011) explained the inhibition of *Listeria monocytogenes* by *Lactococcus lactis* on surfaces by considering the 3D race between the two species. Using a simplified individual-based model approach, they demonstrated that the differences in the growth parameters (lag phase and growth rate) of the two species could explain the observed inhibition of the pathogenic cells. *Lactococcus lactis* cells rapidly formed layers on the mixed community and completely saturated the interface in contact with the nutrient, limiting nutrient access to the pathogen. This example illustrates that part of a biofilm population can be starved within the bulk of the biostructure, even in a very rich environment. In addition, Liu *et al.* (2016) underlined that the specific interactions between species, such as strong or weak cooperation, exploitation or competition, contribute mostly to the spatial organization of biofilms, as these interactions create fitness effects in multispecies biofilms. Taking spatial organization and interspecies interactions within multispecies biofilms into account could increase our understanding of the interactions that take place in agrosystems that use biocontrol agents.

Antibiosis

The production of secondary metabolites by selected organisms is one of the best described mechanisms of

agricultural microbial biocontrol (Ongena *et al*., 2005; Ongena and Jacques, 2008; Khezri *et al*., 2011; Cawoy *et al*., 2014, 2015; Chen *et al*., 2015; Aleti *et al*., 2016; Raza *et al*., 2016; Saravanakumar *et al*., 2016). *Bacillus* genomes contain many genes involved in the production of secondary metabolites, recently compiled in an exhaustive classification of known and putative antimicrobial compounds (Zhao and Kuipers, 2016). Indeed, 4–5% of the genome of *B. subtilis* is allocated to the production of antibiotics and 8.5% of the genome of *B. amyloliquefaciens* FZB42 is allocated to the production of secondary metabolites with antimicrobial properties (Stein, 2005; Chen *et al*., 2009; Zhao and Kuipers, 2016). Many exhibit interesting antibacterial properties (e.g. difficidin), antifungal properties (e.g. bacillomycin D, fengycin and surfactin), or both (e.g. bacilysin) (Ongena and Jacques, 2008; Chen *et al*., 2009; Guo *et al*., 2014; Guo *et al*., 2015; Chowdhury *et al*., 2015; Luo *et al*., 2015; Kröber *et al*., 2016). Most of the studies that have analysed the profile of antimicrobial production have relied on experiments using planktonic laboratory cultures. However, in *B. amyloliquefaciens* FZB42, the genes involved in bacilysin synthesis are overexpressed in biofilms, suggesting that the bacteria have a stronger antagonistic effect in their sessile mode of life (Fig. 3) (Kröber *et al*., 2016). Similarly, in *B. subtilis*, the regulator NtdR controls the expression of the *ntdABC* operon, encoding enzymes involved in the biosynthesis of the antibiotic kanosamine (Inaoka *et al*., 2004; Vetter *et al*., 2013). A global transcriptomic study that compared gene expression of *B. subtilis* in various modes of life showed that this operon is strongly overexpressed in biofilms (Nicolas *et al*., 2012), suggesting the possible involvement of kanosamine in interspecies interactions in plurimicrobial biofilms. Volatile organic compounds (VOCs) can also trigger antimicrobial activity (Khezri *et al*., 2011; Audrain *et al*., 2015; Raza *et al*., 2016). Raza *et al*. (2016) demonstrated that VOCs of *B. amyloliquefaciens* SQR9 inhibited the growth of *Ralstonia solanacearum* on agar medium or in soil. Altogether, these studies show that secondary metabolites with antimicrobial activity can be overproduced (or simply produced) in the biofilm lifestyle, improving antagonistic biocontrol activity. The presence of EPS or amyloid fibres in biofilms can also locally concentrate these molecules and prevent their dilution into the ambient aqueous environment, and thus presumably increase the virulence of biocontrol agents against pathogens in agrosystems (Bianciotto *et al*., 2001; Romero *et al*., 2010; Xu *et al*., 2014; Flemming *et al*., 2016). Previous studies highlighted effects of antimicrobials secreted by one producer on crop protection. Santhanam *et al*. (2015) have also shown that in certain cases, a consortium of different antimicrobial producers is required for optimal plant bioprotection.

Microbial communication

Biofilms are dense, spatially organized communities of microorganisms with extensive forms of social life. They can use specific signalling molecules (autoinducers) that allow them to sense and communicate with the local surrounding populations (Fuqua *et al*., 1994). This quorum sensing (QS) is involved in various biological processes, such as swarming, stress tolerance (pH, antimicrobials, etc.), the production of secondary metabolites, horizontal gene transfer, colonization, biofilm maturation and the synthesis of virulence factors (Fuqua *et al*., 1994; Von Bodman *et al*., 2003). These signalling pathways are widely used in bacteria–bacteria and bacteria–eukaryote associations to regulate and coordinate their interactions. For example, *N*-acylhomoserine lactones (AHL) in Gram-negative bacteria, oligopeptides in Gram-positive bacteria and gamma-butyrolactones in species of the genus *Streptomyces* are autoinducers (Danhorn and Fuqua, 2007). In *Pseudomonas aeruginosa*, QS controls the expression of many bacterial functions. The LasI-LasR QS system, with the autoinducer synthase LasI and the signal receptor LasR, is involved in biofilm maturation and the organization of its 3D structure. A *lasI* mutant can initiate biofilm formation but is unable to form a mature biofilm, suggesting that LasI is involved in the late stages of biofilm development (Davies *et al*., 1998; De Kievit *et al*., 2001). Kavanaugh and Horswill (2016) demonstrated that the *Staphylococci* QS system, *agr*, is involved in biofilm disruption and dispersal.

In the field of biocontrol, it was shown that the protective activity of *Pseudomonas fluorescens* 2P24 on wheat was mainly controlled by the PcoI-PcoR QS system that governs biofilm formation, and not directly by the production of antimicrobial metabolites (Wei and Zhang, 2006). Such social behaviour has been shown to also govern intermicrobial and interkingdom interactions, such as communication interference represented in Fig. 3 or cooperative communication (Zhang and Dong, 2004; Kalia, 2013). For example, *P. aeruginosa* secretes 3-oxo-C12-HSL that affects the growth of *C. albicans* hyphae and inhibits its biofilm formation (Hogan *et al*., 2004). Amyloid fibres of the matrix form aggregates that can act as QS inhibitors by binding QS signalling molecules, and thus locally concentrate these molecules that can reach a 1000-fold higher concentration in the matrix than in planktonic cell environments (Charlton *et al*., 2000; Hense *et al*., 2007; Romero *et al*., 2010; Flemming *et al*., 2016). Other types of molecules can quench or degrade QS signalling molecules of another species (Zhang and Dong, 2004). Indeed, AHL-lactonase of *Bacillus thuringiensis* hinders the accumulation of AHL of *Erwinia carotovora*, thus decreasing the virulence of this bacterium on potatoes (Dong *et al*., 2004). In *Bacillus*,

the lipopeptide surfactin, in addition to its antibiotic properties, can act like a signalling molecule to promote biofilm formation of the other relative *Bacillus* (López *et al*., 2009; Aleti *et al*., 2016). Volatile organic compounds emitted by prokaryotic and eukaryotic microbes are also part of their communication repertoire and can trigger global reprogramming of gene expression of their perceivers (Farag *et al*., 2013; Audrain *et al*., 2015; Raza *et al*., 2016). For example, acetic acid emitted by *B. subtilis* biofilms can promote biofilm formation of other physically separated *B. subtilis* cells and thus act as an important pathway of cell–cell communication (Audrain *et al*., 2015; Chen *et al*., 2015). These communication phenomena specific to biofilms, or amplified in biofilms, could be used to improve biocontrol in agrosystems.

Stress tolerance: adaptability properties and matrix as a protective shield

Bacteria in biofilms exhibit specific physiologies associated with increased tolerance/resistance of the overall community to harsh conditions (Costerton *et al*., 1999; Whiteley *et al*., 2001; Shemesh *et al*., 2007; Bridier *et al*., 2011b). Physiological differences between sessile and planktonic cells are mostly related to differential patterns of gene expression associated with the two modes of life (Whiteley *et al*., 2001; Shemesh *et al*., 2007). Transcriptomic analysis of *Streptococcus mutans*, a bacterium associated with tooth decay, showed that 12% of the genome was differentially expressed in biofilm communities relative to their single-cell homologues. The differentially expressed genes coding for known functions are involved in transport, signalling, metabolism, protein and antimicrobial synthesis (Shemesh *et al*., 2007). Mark *et al.* (2005) evaluated the influence of exudates of two varieties of sugar beets on the transcriptomic profile of *Pseudomonas aeruginosa* PAO1. They showed that the expression of 516 genes was altered in response to one exudates and 451 to the other, and 134 genes responded to both. They found that genes coding for the synthesis of alginate, a major component of the biofilm matrix, were upregulated. These results suggest that *P. aeruginosa* PAO1 forms a biofilm in response to sugar beet exudates. They also showed that the transcriptomic profile of *Pseudomonas aeruginosa* PAO1 in response to exudates is variety dependent. Similarly, Matilla *et al.* (2007) compared the transcriptome profiles of *Pseudomonas putida* KT2440 in the planktonic exponential growth phase, the planktonic stationary growth phase, the sessile form, in sand microcosms and in the rhizosphere. They showed that transcriptomic profile of the planktonic mode of life in the stationary growth phase was the most different from that of the rhizosphere, whereas that of the biofilm lifestyle was more comparable. Indeed, they found that the gene involved in the synthesis of alginate was upregulated in the rhizosphere (Matilla *et al*., 2007; : additional data file). These 'omics' studies confirm the presence of biofilm formation in the rhizosphere or in response to plant exudates. These techniques should be increasingly considered in the study of microbial interactions in agrosystems and extended to metagenomics, metaproteomics and metatranscriptomic approaches as successfully performed in other fields (Blottière *et al*., 2013; Kaul *et al*., 2016).

Other cellular variations can occur during biofilm formation. In the early stages, *Pseudomonas aeruginosa* shows enhanced genetic diversification. Resulting phenotypes vary from cells involved in accelerated biofilm formation to those with enhanced dispersion properties. In the first case, biofilms exhibited pronounced spatial differentiation leading to rough and wrinkled colonies on agar. In the second case, the biofilms showed little spatial organization resulting in small and flat colonies (Boles *et al*., 2004). These differences illustrate the genetic plasticity of cells within a biofilm that enables them to cope with harsh environmental conditions. Stewart and Franklin (2008) also reported the existence of nutrient and oxygen gradients within biofilms creating a stratification of local microenvironments associated with a diversification of cell physiologies (Fig. 3). Population heterogeneity can generate multiple regulatory pathways leading, for example, to the phenomenon of competence in a subpopulation of cells, which coupled with the spatial proximity, facilitates horizontal gene transfer between biofilm cells. This can include the acquisition of plasmids carrying antimicrobial resistance genes (Witte, 2000; Abraham, 2011; Kung and Almeida, 2014; Liu *et al*., 2016). This diversification of cell types in biofilms strongly suggests that the biofilm lifestyle of biocontrol agents enables them to better adapt to, and resist, the hostile conditions encountered in agrosystems (the so-called insurance effects in Boles *et al*., 2004) than their planktonic counterparts.

Biofilms are ubiquitous and subject to harsh conditions, such as the presence of antimicrobials or desiccation. Stewart (2015) recently performed a meta-analysis of the literature from which he proposed that biofilm tolerance to antimicrobials depends neither on the size or chemistry of the antimicrobials nor the composition of the microbial biofilm or the material to which it adheres (Stewart, 2015). Based on his analysis, biofilm-associated tolerance is primarily related to the nature and composition of the biofilm matrix. Indeed, the composition of the matrix creates various meshes within the biofilm leading to a diffusion–reaction limitation that can reduce antimicrobial penetration and local biodisponibility (Fig. 3) (Stewart *et al*., 2001; Stewart and Franklin, 2008; Flemming and Wingender, 2010; Bridier *et al*.,

2011b; Stewart, 2015). Stewart (2015) also stressed that the presence of the matrix and the associated 3D organization renders slow-growth populations less sensitive to certain stresses than their planktonic counterparts.

The matrix also plays a central role in interspecies and interkingdom interactions. *Staphylococcus aureus* and *Candida albicans* are often associated in human diseases, where they form a polymicrobial biofilm (Harriott and Noverr, 2009; Lindsay and Hogan, 2014). This association allows *S. aureus* to resist vancomycin, an antibiotic that is usually efficient against the planktonic form of *S. aureus*. The biofilm of *C. albicans* serves as the backbone of *S. aureus* microcolonies that form on their surface with the matrix of *C. albicans* covering and protecting the cells of *S. aureus* from the action of the antibiotic (Harriott and Noverr, 2009). Other reports have described the protection of *Staphylococcus epidermidis* by *C. albicans* (Adam *et al*., 2002) or of *S. aureus* by *B. subtilis* (Sanchez-Vizuete *et al*., 2015). In the latter case, the authors identified a single gene of the *B. subtilis* NDmed whose disruption suppressed the protective effect. This gene (*ypqP)* is likely involved in the production of matrix exopolysaccharides (Sanchez-Vizuete *et al*., 2015). Non-polysaccharidic components of the matrix can also contribute to the matrix shield; the amphiphilic protein BlsA produced by *B. subtilis* prevents the penetration of biocides by forming a hydrophobic 'raincoat' layer at the biofilm–air interface (Epstein *et al*., 2011; Kobayashi and Iwano, 2012). Biocontrol agents likely benefit from this protective shield on crop surfaces to avoid invasion by aggressive detrimental flora and protect crops.

The direct response of crops to biocontrol agents

Plants can develop natural defence systems against pathogenic microorganisms during their interactions with their environment (biotic and abiotic) (Pieterse and Wees, 2015). Several induced diseases resistance (IDR) mechanisms have been described, including induced systemic resistance (ISR) that is an innate defence mechanism of the plant (Choudhary and Johri, 2009; Pieterse and Wees, 2015) elicited by various environmental stimuli, such as VOCs and QS signalling molecules (Farag *et al*., 2006; Choudhary and Johri, 2009; Matilla *et al*., 2010; Wu *et al*., 2015; Aleti *et al*., 2016). Various stimuli, such as VOCs, QS signals and phytohormones produced and concentrated in the biofilm matrix, can stimulate plant growth, analogous to ISR (Fig. 3) (Espinosa-Urgel *et al*., 2002; Farag *et al*., 2006; Han *et al*., 2006; Spaepen, 2015; Zhang *et al*., 2015; Díaz Herrera *et al*., 2016; Ding *et al*., 2016b). Thus, VOCs, originating from various sources, can induce ISR and plant growth (Yi *et al*., 2009; Farag *et al*., 2013; Audrain *et al*., 2015; Raza *et al*., 2016; Sharifi and Ryu, 2016). These host responses can also be induced by products coming from plant growth-promoting rhizobacteria that have already colonized the root surface, or endophyte colonization of its host (Whipps, 2001; Farag *et al*., 2006; Borriss, 2015; Díaz Herrera *et al*., 2016). The plant growth-promoting rhizobacteria *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a can produce 2,3-butanediol and acetoin on plant roots and promote both plant growth and ISR by eliciting these phenomena (Ryu *et al*., 2003, 2004; Farag *et al*., 2006). Han *et al.* (2006) also showed that the surface area of tobacco leaves increased when they were exposed to 2,3-butanediol secreted by *Pseudomonas chlororaphis* O6 or exposed to the strain itself, even without physical contact. Phytohormones (auxins, cytokinins, gibberellins, abscisic acid and ethylene) are elicitors, which can be produced by bacteria and play a role in promoting plant growth (Spaepen, 2015; Zhang *et al*., 2015). The auxin, indole-3-acetic acid, is produced by *B. amyloliquefaciens* SQR9 and *B. amyloliquefaciens* FZB42 biofilms and promotes the growth of maize and *Lemna minor* (Chen *et al*., 2007; Idris *et al*., 2007; Zhang *et al*., 2015). Endophytes can promote growth of wheat and protect it from *Fusarium graminearum* (Díaz Herrera *et al*., 2016).

Perspectives for sustainable agroecological approaches

Biocontrol mechanisms triggered by biological control agents in agriculture are not yet well understood, and even unknown in certain cases. A single biocontrol agent can use a combination of various biocontrol processes, best described for the strain *B. amyloliquefaciens* FZB42. The use of this bacilli can lead to antagonism, spatial and nutritional competition, antimicrobial production, the stimulation of plant growth and the induction of plant resistance (Timmusk *et al*., 2005; Haggag and Timmusk, 2008; Babalola, 2010; Fan *et al*., 2011; Xu *et al*., 2011; Kröber *et al*., 2014; Chowdhury *et al*., 2015; Kröber *et al*., 2016; Abd El Daim *et al*., 2015). The biofilm mode of life is still poorly taken into account in biocontrol, although it clearly plays a role in agrosystems and governs some of the observed beneficial effects. It would be informative, in the near future, to include phenotypic screening of the ability of strains to form biofilms as a rapid selection criterion of biocontrol agents. Several high-throughput screening tests that could be used for this application are described in the literature (Azeredo *et al*., 2016). Better genetic knowledge of the various cell functions in biofilms will also open doors to selection criteria based on the presence of specific genes involved in important and specific biocontrol functions (Kaul *et al*., 2016).

Invoking biofilm formation as a determinant of biocontrol efficacy could be a new attractive strategy to better control its beneficial effects. This could be achieved, for example, using natural biofilm promoting molecules that trigger the biocontrol agent QS response. In the case of *B. subtilis*, this could be surfactin, VOCs, specific microbial exopolysaccharides or crop extracts (Bais *et al.*, 2004; Chen *et al.*, 2013; Chen *et al.*, 2015; Audrain *et al.*, 2015; Zhou *et al.*, 2016). This effect could also be obtained by adding a second strain (or more) with the ability to stimulate biofilm formation by the initial biocontrol agent, for example through VOC synthesis (Filoche *et al.*, 2004; Audrain *et al.*, 2015; Chen *et al.*, 2015; Figueiredo *et al.*, 2016). Attention should be paid, in this case, to select strains without antagonistic activity against each other or the beneficial action of the biocontrol agent, as previously reported for certain cocktails in the literature (Xu *et al.*, 2011). The benefit of the biofilm mode of life for biocontrol agents could also be obtained using dedicated formulations, as suggested in other areas; for example, the development of new formulas grown as biofilms to orally administer probiotics (e.g. beads of agar or alginates) is under consideration (Rieu *et al.*, 2014). Similarly, a system using bacteria-containing polymersomes, which permits rapid biofilm growth, has been developed for bioremediation to reduce the toxicity of environmental selenium contamination (Barlow *et al.*, 2016).

Increasing evidence, based on available data from the agrosystem and biofilm fields, strongly suggests that the combination of features associated with the 3D biofilm mode of life should be considered when evaluating the performance of biocontrol organisms.

Acknowledgements

This project was supported by the Région Ile-de-France, DIM ASTREA. We thank the MIMA2 imaging platform and J. Deschamps (INRA) for assistance in microscopy. We thank R. Borriss and R. Zhang for providing the *Bacillus amyloliquefaciens* FZB42 and SQR9 strains respectively. Finally, we warmly thank R. Védie and T. Rousseau from the French Mushroom Centre (Centre Technique du Champignon, Distré) for helpful discussions, and Alex Edelman and Associates for English revision of the manuscript.

Conflict of interest

None declared.

References

Abd El Daim, I., Häggblom, P., Karlsson, M., Stenström, E. and Timmusk, S. (2015) *Paenibacillus polymyxa* A26 Sfp-type PPTase inactivation limits bacterial antagonism against *Fusarium graminearum* but not of *F. culmorum* in kernel assay. *Front Plant Sci* **6,** 368.

Abdian, P. and Zorreguieta, A. (2016) Extracellular factors involved in biofilm matrix formation by Rhizobia. In *The Perfect Slime – Microbial Extracellular Polymeric Substances (EPS).* Flemming, H.-C., Neu, T.R. and Wingender, J. (eds). London: IWA Publishing, pp. 227–247.

Abraham, W.-R. (2011) Megacities as sources for pathogenic bacteria in rivers and their fate downstream. *Int J Microbiol* **2011:** 211–215.

Adam, B., Baillie, G.S., and Douglas, L.J. (2002) Mixed species biofilms of *Candida albicans* and *Staphylococcus epidermidis*. *J Med Microbiol* **51:** 344–349.

Ahn, S.-J., Yang, C.-H., and Cooksey, D.A. (2007) *Pseudomonas putida* 06909 genes expressed during colonization on mycelial surfaces and phenotypic characterization of mutants. *J Appl Microbiol* **103:** 120–132.

Aktar, M.W., Sengupta, D., and Chowdhury, A. (2009) Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip Toxicol* **2:** 1–12.

Aleti, G., Lehner, S., Bacher, M., Compant, S., Nikolic, B., Plesko, M., *et al.* (2016) Surfactin variants mediate species-specific biofilm formation and root colonization in *Bacillus*. *Environ Microbiol* **18:** 2634–2645.

Anonymous. (1999). Killer environment. *Environ Health Perspect* **107,** A62–A63.

Aparna, M.S., and Yadav, S. (2008) Biofilms: microbes and disease. *Braz J Infect Dis* **12:** 526–530.

Audrain, B., Farag, M.A., Ryu, C.-M., and Ghigo, J.-M. (2015) Role of bacterial volatile compounds in bacterial biology. *FEMS Microbiol Rev* **39:** 222–233.

Azeredo, J., Azevedo, N.F., Briandet, R., Cerca, N., Coenye, T., Costa, A.R., *et al.* (2016) Critical review on biofilm methods. *Crit Rev Microbiol* [Epub ahead of print]. doi:10.1080/1040841X.2016.1208146.

Babalola, O.O. (2010) Beneficial bacteria of agricultural importance. *Biotechnol Lett* **32:** 1559–1570.

Bais, H.P., Fall, R., and Vivanco, J.M. (2004) Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiol* **134:** 307–319.

Barlow, J., Gozzi, K., Kelley, C.P., Geilich, B.M., Webster, T.J., Chai, Y., *et al.* (2016) High throughput microencapsulation of *Bacillus subtilis* in semi-permeable biodegradable polymersomes for selenium remediation. *Appl Microbiol Biotechnol* **101:** 455–464.

Bianciotto, V., Andreotti, S., Balestrini, R., Bonfante, P., and Perotto, S. (2001) Mucoid mutants of the biocontrol strain *Pseudomonas fluorescens* CHA0 show increased ability in biofilm formation on mycorrhizal and nonmycorrhizal carrot roots. *Mol Plant-Microbe Interact MPMI* **14:** 255–260.

Blanchette, K.A., and Orihuela, C.J. (2012) Future perspective on host-pathogen interactions during bacterial biofilm formation within the nasopharynx. *Future Microbiol* **7:** 227–239.

Blottière, H.M., de Vos, W.M., Ehrlich, S.D., and Doré, J. (2013) Human intestinal metagenomics: state of the art and future. *Curr Opin Microbiol* **16:** 232–239.

Bogino, P.C., Oliva, M.de.las.M., Sorroche, F.G. and Giordano, W. (2013) The role of bacterial biofilms and surface

components in plant-bacterial associations. *Int J Mol Sci* **14,** 15838–15859.
Boles, B.R., Thoendel, M., and Singh, P.K. (2004) Self-generated diversity produces 'insurance effects' in biofilm communities. *Proc Natl Acad Sci USA* **101:** 16630–16635.
Borriss, R. (2015) *Bacillus*, a plant-beneficial bacterium. In *Princ Plant-Microbe Interact*. Lugtenberg, B. (ed.). Cham, Switzerland: Springer International Publishing, pp. 379–391.
Branda, S.S., González-Pastor, J.E., Dervyn, E., Ehrlich, S.D., Losick, R., and Kolter, R. (2004) Genes involved in formation of structured multicellular communities by *Bacillus subtilis*. *J Bacteriol* **186:** 3970–3979.
Breitenbach, R., Toepel, J., Dementyeva, P., Knabe, N. and Gorbushina, A. (2016) Snapshots of fungal extracellular matrices. In *The Perfect Slime – Microbial Extracellular Polymeric Substances (EPS)*. Flemming, H.-C., Neu, T.R. and Wingender, J. (eds). London: IWA Publishing, pp. 269–299.
Breugelmans, P., Barken, K.B., Tolker-Nielsen, T., Hofkens, J., Dejonghe, W., and Springael, D. (2008) Architecture and spatial organization in a triple-species bacterial biofilm synergistically degrading the phenylurea herbicide linuron. *FEMS Microbiol Ecol* **64:** 271–282.
Bridier, A., Briandet, R., Thomas, V., and Dubois-Brissonnet, F. (2011a) Resistance of bacterial biofilms to disinfectants: a review. *Biofouling* **27:** 1017–1032.
Bridier, A., Dubois-Brissonnet, F., Greub, G., Thomas, V., and Briandet, R. (2011b) Dynamics of the action of biocides in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother* **55:** 2648–2654.
Cairns, L.S., Hobley, L., and Stanley-Wall, N.R. (2014) Biofilm formation by *Bacillus subtilis*: new insights into regulatory strategies and assembly mechanisms. *Mol Microbiol* **93:** 587–598.
Cawoy, H., Mariutto, M., Henry, G., Fisher, C., Vasilyeva, N., Thonart, P., *et al.* (2014) Plant defence stimulation by natural isolates of *Bacillus* depends on efficient surfactin production. *Mol Plant-Microbe Interact MPMI* **27:** 87–100.
Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., and Ongena, M. (2015) Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. *Microb Biotechnol* **8:** 281–295.
Charlton, T.S., de Nys, R., Netting, A., Kumar, N., Hentzer, M., Givskov, M., and Kjelleberg, S. (2000) A novel and sensitive method for the quantification of N-3-oxoacyl homoserine lactones using gas chromatography-mass spectrometry: application to a model bacterial biofilm. *Environ Microbiol* **2:** 530–541.
Chen, X.H., Koumoutsi, A., Scholz, R., Eisenreich, A., Schneider, K., Heinemeyer, I., *et al.* (2007) Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nat Biotechnol* **25:** 1007–1014.
Chen, X.H., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Süssmuth, R., *et al.* (2009) Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. *J Biotechnol* **140:** 27–37.
Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R., and Guo, J. (2013) Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environ Microbiol* **15:** 848–864.
Chen, Y., Gozzi, K., Yan, F. and Chai, Y. (2015) Acetic acid acts as a volatile signal to stimulate bacterial biofilm formation. *mBio* **6,** e00392.
Chi, M., Li, G., Liu, Y., Liu, G., Li, M., Zhang, X., *et al.* (2015) Increase in antioxidant enzyme activity, stress tolerance and biocontrol efficacy of *Pichia kudriavzevii* with the transition from a yeast-like to biofilm morphology. *Biol Control* **90:** 113–119.
Choudhary, D.K., and Johri, B.N. (2009) Interactions of *Bacillus spp.* and plants – with special reference to induced systemic resistance (ISR). *Microbiol Res* **164:** 493–513.
Chowdhury, S.P., Hartmann, A., Gao, X., and Borriss, R. (2015) Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 - a review. *Front Microbiol* **6:** 780.
Costerton, J.W., Geesey, G.G., and Cheng, K.J. (1978) How bacteria stick. *Sci Am* **238:** 86–95.
Costerton, J.W., Stewart, P.S., and Greenberg, E.P. (1999) Bacterial biofilms: a common cause of persistent infections. *Science* **284:** 1318–1322.
Danhorn, T., and Fuqua, C. (2007) Biofilm formation by plant-associated bacteria. *Annu Rev Microbiol* **61:** 401–422.
Davey, M.E. and O'toole, G.A. (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev* **64,** 847–867.
Davies, D. (2003) Understanding biofilm resistance to antibacterial agents. *Nat Rev Drug Discov* **2:** 114–122.
Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., and Greenberg, E.P. (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* **280:** 295–298.
De Kievit, T.R., Gillis, R., Marx, S., Brown, C., and Iglewski, B.H. (2001) Quorum-sensing genes in *Pseudomonas aeruginosa* biofilms: their role and expression patterns. *Appl Environ Microbiol* **67:** 1865–1873.
De la Fuente, M., Vidal, J.M., Miranda, C.D., González, G., and Urrutia, H. (2013) Inhibition of *Flavobacterium psychrophilum* biofilm formation using a biofilm of the antagonist *Pseudomonas fluorescens* FF48. *SpringerPlus* **2:** 176.
Díaz Herrera, S., Grossi, C., Zawoznik, M., and Groppa, M.D. (2016) Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of *Fusarium graminearum*. *Microbiol Res* **186–187:** 37–43.
Ding, C., Yang, Z., Wang, J., Liu, X., Cao, Y., Pan, Y., *et al.* (2016a) Prevalence of *Pseudomonas aeruginosa* and antimicrobial-resistant *Pseudomonas aeruginosa* in patients with pneumonia in mainland China: a systematic review and meta-analysis. *Int J Infect Dis* **49:** 119–126.
Ding, L., Cao, J., Duan, Y., Li, J., Yang, Y., Yang, G., and Zhou, Y. (2016b) Proteomic and physiological responses of *Arabidopsis thaliana* exposed to salinity stress and N-acyl-homoserine lactone. *Physiol Plant* **158:** 414–434.
Dong, Y.-H., Zhang, X.-F., Xu, J.-L., and Zhang, L.-H. (2004) Insecticidal *Bacillus thuringiensis* silences *Erwinia*

carotovora virulence by a new form of microbial antagonism, signal interference. *Appl Environ Microbiol* **70:** 954–960.

Epstein, A.K., Pokroy, B., Seminara, A., and Aizenberg, J. (2011) Bacterial biofilm shows persistent resistance to liquid wetting and gas penetration. *Proc Natl Acad Sci* **108:** 995–1000.

Espinosa-Urgel, M., Kolter, R., and Ramos, J.-L. (2002) Root colonization by *Pseudomonas putida*: love at first sight. *Microbiology* **148:** 341–343.

Fan, B., Chen, X.H., Budiharjo, A., Bleiss, W., Vater, J., and Borriss, R. (2011) Efficient colonization of plant roots by the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42, engineered to express green fluorescent protein. *J Biotechnol* **151:** 303–311.

Farag, M.A., Ryu, C.-M., Sumner, L.W., and Paré, P.W. (2006) GC–MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. *Phytochemistry* **67:** 2262–2268.

Farag, M.A., Zhang, H., and Ryu, C.-M. (2013) Dynamic chemical communication between plants and bacteria through airborne signals: induced resistance by bacterial volatiles. *J Chem Ecol* **39:** 1007–1018.

Figueiredo, M.do.V.B., Bonifacio, A., Rodrigues, A.C., de Araujo, F.F. and Stamford, N.P. (2016) Beneficial microorganisms: current challenge to increase crop performance. In *Bioformulations Sustain Agric*. Arora, N.K., Mehnaz, S. and Balestrini, R. (eds). New Delhi: Springer India, pp. 53–70.

Filoche, S.K., Anderson, S.A., and Sissons, C.H. (2004) Biofilm growth of *Lactobacillus* species is promoted by *Actinomyces* species and *Streptococcus mutans*. *Oral Microbiol Immunol* **19:** 322–326.

Flemming, H.-C., and Wingender, J. (2010) The biofilm matrix. *Nat Rev Microbiol* **8:** 623–633.

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., and Kjelleberg, S. (2016) Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol* **14:** 563–575.

Fuqua, W.C., Winans, S.C., and Greenberg, E.P. (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* **176:** 269–275.

van Gestel, J., Vlamakis, H., and Kolter, R. (2015) From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. *PLoS Biol* **13:** doi:10.1371/journal.pbio.1002141.

Gulati, M., and Nobile, C.J. (2016) *Candida albicans* biofilms: development, regulation, and molecular mechanisms. *Microbes Infect* **18:** 310–321.

Guo, Q., Dong, W., Li, S., Lu, X., Wang, P., Zhang, X., *et al.* (2014) Fengycin produced by *Bacillus subtilis* NCD-2 plays a major role in biocontrol of cotton seedling damping-off disease. *Microbiol Res* **169:** 533–540.

Guo, S., Li, X., He, P., Ho, H., Wu, Y., and He, Y. (2015) Whole-genome sequencing of *Bacillus subtilis* XF-1 reveals mechanisms for biological control and multiple beneficial properties in plants. *J Ind Microbiol Biotechnol* **42:** 925–937.

Haagensen, J.A.J., Hansen, S.K., Christensen, B.B., Pamp, S.J., and Molin, S. (2015) Development of spatial distribution patterns by biofilm-cells. *Appl Environ Microbiol* **81:** 6120–6128.

Habimana, O., Guillier, L., Kulakauskas, S., and Briandet, R. (2011) Spatial competition with *Lactococcus lactis* in mixed-species continuous-flow biofilms inhibits *Listeria monocytogenes* growth. *Biofouling* **27:** 1065–1072.

Haggag, W.M., and Timmusk, S. (2008) Colonization of peanut roots by biofilm-forming *Paenibacillus polymyxa* initiates biocontrol against crown rot disease. *J Appl Microbiol* **104:** 961–969.

Han, S.H., Lee, S.J., Moon, J.H., Park, K.H., Yang, K.Y., Cho, B.H., *et al.* (2006) GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. *Mol Plant Microbe Interact* **19:** 924–930.

Harriott, M.M., and Noverr, M.C. (2009) *Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. *Antimicrob Agents Chemother* **53:** 3914–3922.

Hazell, P., and Wood, S. (2008) Drivers of change in global agriculture. *Philos Trans R Soc Lond B Biol Sci* **363:** 495–515.

He, X., Wang, J., Abdoli, L., and Li, H. (2016) Mg(2+)/Ca(2+) promotes the adhesion of marine bacteria and algae and enhances following biofilm formation in artificial seawater. *Colloids Surf B Biointerfaces* **146:** 289–295.

Hense, B.A., Kuttler, C., Müller, J., Rothballer, M., Hartmann, A., and Kreft, J.-U. (2007) Does efficiency sensing unify diffusion and quorum sensing? *Nat Rev Microbiol* **5:** 230–239.

Hogan, D.A., Vik, A., and Kolter, R. (2004) A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. *Mol Microbiol* **54:** 1212–1223.

Horrigan, L., Lawrence, R.S., and Walker, P. (2002) How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environ Health Perspect* **110:** 445–456.

Houry, A., Gohar, M., Deschamps, J., Tischenko, E., Aymerich, S., Gruss, A., and Briandet, R. (2012) Bacterial swimmers that infiltrate and take over the biofilm matrix. *Proc Natl Acad Sci USA* **109:** 13088–13093.

Idris, E.E., Iglesias, D.J., Talon, M., and Borriss, R. (2007) Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Mol Plant Microbe Interact* **20:** 619–626.

Inaoka, T., Takahashi, K., Yada, H., Yoshida, M., and Ochi, K. (2004) RNA polymerase mutation activates the production of a dormant antibiotic 3,3′-neotrehalosadiamine via an autoinduction mechanism in *Bacillus subtilis*. *J Biol Chem* **279:** 3885–3892.

de Jong, W., Wösten, H.A.B., Dijkhuizen, L., and Claessen, D. (2009) Attachment of *Streptomyces coelicolor* is mediated by amyloidal fimbriae that are anchored to the cell surface via cellulose. *Mol Microbiol* **73:** 1128–1140.

Jordan, K., Dalmasso, M., Zentek, J., Mader, A., Bruggeman, G., Wallace, J., *et al.* (2014) Microbes versus microbes: control of pathogens in the food chain. *J Sci Food Agric* **94:** 3079–3089.

Kalia, V.C. (2013) Quorum sensing inhibitors: an overview. *Biotechnol Adv* **31:** 224–245.

Kaul, S., Sharma, T. and K Dhar, M. (2016) "Omics" tools for better understanding the plant-endophyte interactions. *Front Plant Sci* **7,** 955.

Kavanaugh, J.S., and Horswill, A.R. (2016) Impact of environmental cues on Staphylococcal quorum-sensing and biofilm development. *J Biol Chem* **24:** 12556–12564.

Kearns, D.B., Chu, F., Rudner, R., and Losick, R. (2004) Genes governing swarming in *Bacillus subtilis* and evidence for a phase variation mechanism controlling surface motility. *Mol Microbiol* **52:** 357–369.

Khezri, M., Ahmadzadeh, M., Jouzani, G.S., Behboudi, K., Ahangaran, A., Mousivand, M., and Rahimian, H. (2011) Characterization of some biofilm-forming *Bacillus subtilis* strains and evaluation of their biocontrol potential against *Fusarium culmorum*. *J Plant Pathol* **93:** 373–382.

Kobayashi, K., and Iwano, M. (2012) BslA (YuaB) forms a hydrophobic layer on the surface of *Bacillus subtilis* biofilms. *Mol Microbiol* **85:** 51–66.

Kröber, M., Wibberg, D., Grosch, R., Eikmeyer, F., Verwaaijen, B., Chowdhury, S.P., *et al.* (2014) Effect of the strain *Bacillus amyloliquefaciens* FZB42 on the microbial community in the rhizosphere of lettuce under field conditions analyzed by whole metagenome sequencing. *Front Microbiol* **5:** 252.

Kröber, M., Verwaaijen, B., Wibberg, D., Winkler, A., Pühler, A., and Schlüter, A. (2016) Comparative transcriptome analysis of the biocontrol strain *Bacillus amyloliquefaciens* FZB42 as response to biofilm formation analyzed by RNA sequencing. *J Biotechnol* **231:** 212–223.

Kung, S.H., and Almeida, R.P.P. (2014) Biological and genetic factors regulating natural competence in a bacterial plant pathogen. *Microbiol Read Engl* **160:** 37–46.

Lam, J., Chan, R., Lam, K., and Costerton, J.W. (1980) Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. *Infect Immun* **28:** 546–556.

Li, P., Pu, X., Feng, B., Yang, Q., Shen, H., Zhang, J., and Lin, B. (2015) *FocVel1* influences asexual production, filamentous growth, biofilm formation, and virulence in *Fusarium oxysporum* f. sp. *cucumerinum*. *Front Plant Sci* **6:** 312.

Lindsay, A.K., and Hogan, D.A. (2014) *Candida albicans*: molecular interactions with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Fungal Biol Rev* **28:** 85–96.

Liu, J., Prindle, A., Humphries, J., Gabalda-Sagarra, M., Asally, M., Lee, D.D., *et al.* (2015) Metabolic codependence gives rise to collective oscillations within biofilms. *Nature* **523:** 550–554.

Liu, W., Røder, H.L., Madsen, J.S., Bjarnsholt, T., Sørensen, S.J., and Burmølle, M. (2016) Interspecific bacterial interactions are reflected in multispecies biofilm spatial organization. *Front Microbiol* **7:** 1366.

López, D., and Kolter, R. (2010) Extracellular signals that define distinct and coexisting cell fates in *Bacillus subtilis*. *FEMS Microbiol Rev* **34:** 134–149.

López, D., Vlamakis, H., Losick, R., and Kolter, R. (2009) Paracrine signaling in a bacterium. *Genes Dev* **23:** 1631–1638.

Luo, C., Zhou, H., Zou, J., Wang, X., Zhang, R., Xiang, Y., and Chen, Z. (2015) Bacillomycin L and surfactin contribute synergistically to the phenotypic features of *Bacillus subtilis* 916 and the biocontrol of rice sheath blight induced by *Rhizoctonia solani*. *Appl Microbiol Biotechnol* **99:** 1897–1910.

Mark, G.L., Dow, J.M., Kiely, P.D., Higgins, H., Haynes, J., Baysse, C., *et al.* (2005) Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. *Proc Natl Acad Sci USA* **102:** 17454–17459.

Matilla, M.A., Espinosa-Urgel, M., Rodríguez-Herva, J.J., Ramos, J.L., and Ramos-González, M.I. (2007) Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. *Genome Biol* **8:** R179.

Matilla, M.A., Ramos, J.L., Bakker, P.A.H.M., Doornbos, R., Badri, D.V., Vivanco, J.M., and Ramos-González, M.I. (2010) *Pseudomonas putida* KT2440 causes induced systemic resistance and changes in *Arabidopsis* root exudation. *Environ Microbiol Rep* **2:** 381–388.

Mielich-Süss, B., and Lopez, D. (2015) Molecular mechanisms involved in *Bacillus subtilis* biofilm formation. *Environ Microbiol* **17:** 555–565.

Mitchell, K.F., Zarnowski, R., and Andes, D.R. (2016) Fungal super glue: the biofilm matrix and its composition, assembly, and functions. *PLoS Pathog* **12:** e1005828.

Mitri, S., Xavier, J.B., and Foster, K.R. (2011) Social evolution in multispecies biofilms. *Proc Natl Acad Sci USA* **108:** 10839–10846.

Mora, I., Cabrefiga, J., and Montesinos, E. (2015) Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated *Bacillus* against phytopathogenic bacteria. *PLoS ONE* **10:** e0127738.

Morris, C.E., and Monier, J.-M. (2003) The ecological significance of biofilm formation by plant-associated bacteria. *Annu Rev Phytopathol* **41:** 429–453.

National Institutes of Health (NIH). (2002) NIH guide: research on microbial biofilms. URL https://grants.nih.gov/grants/guide/pa-files/PA-03-047.html

Nicolas, P., Mäder, U., Dervyn, E., Rochat, T., Leduc, A., Pigeonneau, N., *et al.* (2012) Condition-dependent transcriptome reveals high-level regulatory architecture in *Bacillus subtilis*. *Science* **335:** 1103–1106.

Oerke, E.-C. (1999) Estimated crop losses due to pathogens, animal pests and weeds. *Crop production and Crop Protection*. Amsterdam: Elsevier, pp. 72–741.

Oerke, E.-C. (2006) Crop losses to pests. *J Agric Sci* **144:** 31–43.

Ongena, M., and Jacques, P. (2008) *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiol* **16:** 115–125.

Ongena, M., Jacques, P., Touré, Y., Destain, J., Jabrane, A., and Thonart, P. (2005) Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of *Bacillus subtilis*. *Appl Microbiol Biotechnol* **69:** 29–38.

O'Toole, G., Kaplan, H.B., and Kolter, R. (2000) Biofilm formation as microbial development. *Annu Rev Microbiol* **54:** 49–79.

Pal, K.K. and McSpadden Gardener, B. (2006) Biological control of plant pathogens. *Plant Health Instr* doi:10.1094/PHI-A-2006-1117-02.

Pandin, C., Caroff, M., and Condemine, G. (2016) Antimicrobial peptide resistance genes in the plant pathogen *Dickeya dadantii*. *Appl Environ Microbiol* **82:** 6423–6430.

Pieterse, C.M.J. and Wees, S.C.M.V. (2015) Induced disease resistance. In *Princ Plant-Microbe Interact*. Lugtenberg, B. (ed.). Cham, Switzerland: Springer International Publishing, pp. 123–133.

Prigent-Combaret, C., Zghidi-Abouzid, O., Effantin, G., Lejeune, P., Reverchon, S., and Nasser, W. (2012) The nucleoid-associated protein Fis directly modulates the synthesis of cellulose, an essential component of pellicle–biofilms in the phytopathogenic bacterium *Dickeya dadantii*. *Mol Microbiol* **86:** 172–186.

Prindle, A., Liu, J., Asally, M., Ly, S., Garcia-Ojalvo, J., and Suel, G.M. (2015) Ion channels enable electrical communication in bacterial communities. *Nature* **527:** 59–63.

Pu, L., Jingfan, F., Kai, C., Chao-an, L., and Yunjiang, C. (2014) Phenylethanol promotes adhesion and biofilm formation of the antagonistic yeast *Kloeckera apiculata* for the control of blue mold on citrus. *FEMS Yeast Res* **14:** 536–546.

Rajendran, A., and Hu, B. (2016) Mycoalgae biofilm: development of a novel platform technology using algae and fungal cultures. *Biotechnol Biofuels* **9:** 112.

Ramey, B.E., Koutsoudis, M., von Bodman, S.B., and Fuqua, C. (2004) Biofilm formation in plant-microbe associations. *Curr Opin Microbiol* **7:** 602–609.

Raza, W., Ling, N., Yang, L., Huang, Q., and Shen, Q. (2016) Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. *Sci Rep* **6:** 24856.

Rendueles, O., and Ghigo, J.-M. (2015) Mechanisms of competition in biofilm communities. *Microbiol Spectr* **3:** doi:10.1128/microbiolspec.MB-0009-2014.

Rieu, A., Aoudia, N., Jego, G., Chluba, J., Yousfi, N., Briandet, R., *et al.* (2014) The biofilm mode of life boosts the anti-inflammatory properties of *Lactobacillus*. *Cell Microbiol* **16:** 1836–1853.

Romero, D., Aguilar, C., Losick, R., and Kolter, R. (2010) Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc Natl Acad Sci* **107:** 2230–2234.

Romero, F.M., Marina, M., and Pieckenstain, F.L. (2016) Novel components of leaf bacterial communities of field-grown tomato plants and their potential for plant growth promotion and biocontrol of tomato diseases. *Res Microbiol* **167:** 222–233.

Ryu, C.-M., Farag, M.A., Hu, C.-H., Reddy, M.S., Wei, H.-X., Paré, P.W., and Kloepper, J.W. (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proc Natl Acad Sci USA* **100:** 4927–4932.

Ryu, C.-M., Farag, M.A., Hu, C.-H., Reddy, M.S., Kloepper, J.W., and Paré, P.W. (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol* **134:** 1017–1026.

Sanchez-Vizuete, P., Le Coq, D., Bridier, A., Herry, J.-M., Aymerich, S., and Briandet, R. (2015) Identification of *ypqP* as a new *Bacillus subtilis* biofilm determinant that mediates the protection of *Staphylococcus aureus* against antimicrobial agents in mixed-species communities. *Appl Environ Microbiol* **81:** 109–118.

Sang, M.K., and Kim, K.D. (2014) Biocontrol activity and root colonization by *Pseudomonas corrugata* strains CCR04 and CCR80 against *Phytophthora* blight of pepper. *Biocontrol* **59:** 437–448.

Santhanam, R., Luu, V.T., Weinhold, A., Goldberg, J., Oh, Y., and Baldwin, I.T. (2015) Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. *Proc Natl Acad Sci USA* **112:** E5013–E5020.

Saravanakumar, K., Yu, C., Dou, K., Wang, M., Li, Y., and Chen, J. (2016) Synergistic effect of *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum*. *Biol Control* **94:** 37–46.

Savary, S., Ficke, A., Aubertot, J.-N., and Hollier, C. (2012) Crop losses due to diseases and their implications for global food production losses and food security. *Food Secur* **4:** 519–537.

Selin, C., Habibian, R., Poritsanos, N., Athukorala, S.N.P., Fernando, D., and de Kievit, T.R. (2010) Phenazines are not essential for *Pseudomonas chlororaphis* PA23 biocontrol of *Sclerotinia sclerotiorum*, but do play a role in biofilm formation. *FEMS Microbiol Ecol* **71:** 73–83.

Sharifi, R., and Ryu, C.-M. (2016) Are bacterial volatile compounds poisonous odors to a fungal pathogen *Botrytis cinerea*, alarm signals to *Arabidopsis* seedlings for eliciting induced resistance, or both? *Front Microbiol* **7:** 196.

Shemesh, M., Tam, A., and Steinberg, D. (2007) Differential gene expression profiling of *Streptococcus mutans* cultured under biofilm and planktonic conditions. *Microbiology* **153:** 1307–1317.

Sheppard, D.C., and Howell, P.L. (2016) Biofilm exopolysaccharides of pathogenic fungi: lessons from bacteria. *J Biol Chem* **291:** 12529–12537.

Shirazi, F., Ferreira, J.A.G., Stevens, D.A., Clemons, K.V., and Kontoyiannis, D.P. (2016) Biofilm filtrates of *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients inhibit preformed *Aspergillus fumigatus* biofilms *via* apoptosis. *PLoS ONE* **11:** e0150155.

Spaepen, S. (2015) Plant hormones produced by microbes. In *Princ Plant-Microbe Interact*. Lugtenberg, B. (ed.). Cham, Switzerland: Springer International Publishing, pp. 247–256.

Steddom, K., Menge, J.A., Crowley, D., and Borneman, J. (2002) Effect of repetitive applications of the biocontrol bacterium *Pseudomonas putida* 06909-rif/nal on citrus soil microbial communities. *Phytopathology* **92:** 857–862.

Stein, T. (2005) *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Mol Microbiol* **56:** 845–857.

Stewart, P.S. (2015) Antimicrobial tolerance in biofilms. *Microbiol Spectr* **3.** doi:10.1128/microbiolspec.MB-0010-2014.

Stewart, P.S., and Franklin, M.J. (2008) Physiological heterogeneity in biofilms. *Nat Rev Microbiol* **6:** 199–210.

Stewart, P.S., Rayner, J., Roe, F., and Rees, W.M. (2001) Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. *J Appl Microbiol* **91:** 525–532.

Sundh, I., and Melin, P. (2010) Safety and regulation of yeasts used for biocontrol or biopreservation in the food or feed chain. *Antonie Van Leeuwenhoek* **99:** 113–119.

Sutherland, I.W. (2001) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* **147:** 3–9.

Teng, P.S. (1987) *Crop Loss Assessment and Pest Management*. St Paul: APS press. American Phytopathological Society, p. 370.

Teng, P.S. and Krupa, S.V. (1980) Assessment of losses which constrain production and crop improvement in agriculture and forestry: proceedings of the E.C. Stakman commemorative symposium, University of Minnesota. Department of Plant Pathology, 327.

Timmusk, S., Grantcharova, N., and Wagner, E.G.H. (2005) *Paenibacillus polymyxa* invades plant roots and forms biofilms. *Appl Environ Microbiol* **71:** 7292–7300.

Triveni, S., Prasanna, R., and Saxena, A.K. (2012) Optimization of conditions for in vitro development of *Trichoderma viride*-based biofilms as potential inoculants. *Folia Microbiol (Praha)* **57:** 431–437.

Triveni, S., Prasanna, R., Kumar, A., Bidyarani, N., Singh, R., and Saxena, A.K. (2015) Evaluating the promise of *Trichoderma* and *Anabaena* based biofilms as multifunctional agents in *Macrophomina phaseolina*-infected cotton crop. *Biocontrol Sci Technol* **25:** 656–670.

Verhamme, D.T., Kiley, T.B., and Stanley-Wall, N.R. (2007) DegU co-ordinates multicellular behaviour exhibited by *Bacillus subtilis*. *Mol Microbiol* **65:** 554–568.

Vetter, N.D., Langill, D.M., Anjum, S., Boisvert-Martel, J., Jagdhane, R.C., Omene, E., *et al.* (2013) A previously unrecognized kanosamine biosynthesis pathway in *Bacillus subtilis*. *J Am Chem Soc* **135:** 5970–5973.

Vlamakis, H., Aguilar, C., Losick, R., and Kolter, R. (2008) Control of cell fate by the formation of an architecturally complex bacterial community. *Genes Dev* **22:** 945–953.

Vlamakis, H., Chai, Y., Beauregard, P., Losick, R., and Kolter, R. (2013) Sticking together: building a biofilm the *Bacillus subtilis* way. *Nat Rev Microbiol* **11:** 157–168.

Von Bodman, S.B., Bauer, W.D., and Coplin, D.L. (2003) Quorum sensing in plant-pathogenic bacteria. *Annu Rev Phytopathol* **41:** 455–482.

Wang, X., Koehler, S.A., Wilking, J.N., Sinha, N.N., Cabeen, M.T., Srinivasan, S., *et al.* (2016) Probing phenotypic growth in expanding *Bacillus subtilis* biofilms. *Appl Microbiol Biotechnol* **100:** 4607–4615.

Wei, H.-L., and Zhang, L.-Q. (2006) Quorum-sensing system influences root colonization and biological control ability in *Pseudomonas fluorescens* 2P24. *Antonie Van Leeuwenhoek* **89:** 267–280.

Whipps, J.M. (2001) Microbial interactions and biocontrol in the rhizosphere. *J Exp Bot* **52:** 487–511.

Whiteley, M., Bangera, M.G., Bumgarner, R.E., Parsek, M.R., Teitzel, G.M., Lory, S., and Greenberg, E.P. (2001) Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature* **413:** 860–864.

Witte, W. (2000) Ecological impact of antibiotic use in animals on different complex microflora: environment. *Int J Antimicrob Agents* **14:** 321–325.

Wu, K., Fang, Z., Guo, R., Pan, B., Shi, W., Yuan, S., *et al.* (2015) Pectin enhances bio-control efficacy by inducing colonization and secretion of secondary metabolites by *Bacillus amyloliquefaciens* SQY 162 in the rhizosphere of tobacco. *PLoS ONE* **10:** e0127418.

Xu, X.-M., Jeffries, P., Pautasso, M., and Jeger, M.J. (2011) Combined use of biocontrol agents to manage plant diseases in theory and practice. *Phytopathology* **101:** 1024–1031.

Xu, Z., Zhang, R., Wang, D., Qiu, M., Feng, H., Zhang, N., and Shen, Q. (2014) Enhanced control of cucumber wilt disease by *Bacillus amyloliquefaciens* SQR9 by altering the regulation of Its DegU phosphorylation. *Appl Environ Microbiol* **80:** 2941–2950.

Yi, H.-S., Heil, M., Adame-Álvarez, R.M., Ballhorn, D.J., and Ryu, C.-M. (2009) Airborne induction and priming of plant defenses against a bacterial pathogen. *Plant Physiol* **151:** 2152–2161.

Zarnowski, R., Westler, W.M., Lacmbouh, G.A., Marita, J.M., Bothe, J.R., Bernhardt, J., *et al.* (2014) Novel entries in a fungal biofilm matrix encyclopedia. *mBio* **5,** e01333–14.

Zeriouh, H., de Vicente, A., Pérez-García, A., and Romero, D. (2014) Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. *Environ Microbiol* **16:** 2196–2211.

Zhang, L.-H., and Dong, Y.-H. (2004) Quorum sensing and signal interference: diverse implications. *Mol Microbiol* **53:** 1563–1571.

Zhang, N., Yang, D., Wang, D., Miao, Y., Shao, J., Zhou, X., *et al.* (2015) Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates. *BMC Genom* **16:** 685.

Zhao, X., and Kuipers, O.P. (2016) Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. *BMC Genom* **17:** 882.

Zhou, H., Luo, C., Fang, X., Xiang, Y., Wang, X., Zhang, R., and Chen, Z. (2016) Loss of *gltb* inhibits biofilm formation and biocontrol efficiency of *Bacillus subtilis* Bs916 by altering the production of γ-polyglutamate and three lipopeptides. *PLoS ONE* **11:** 1–20.

PRESENTATION DES RESULTATS

Chapitre 1: Pourquoi *Bacillus subtilis* QST713 utilisé pour la bioprotection d'*Agaricus bisporus* est un agent de biocontrôle efficace?

Préambule

Afin de déterminer l'implication de la formation de biofilm et de la synthèse d'antimicrobiens par *B. subtilis* dans les mécanismes de bioprotection s'exerçant dans le pathosystème *A. bisporus*/*T. aggressivum*/*B. subtilis* QST713, une immersion dans la mécanistique de formation de biofilm par *B. subtilis* est tout d'abord nécessaire.

Pour la compréhension des mécanismes moléculaires régissant la formation de biofilms et leurs régulations, *B. subtilis* est un des modèles bactériens le plus documenté actuellement. Le passage de l'état « planctonique » à l'état « biofilm » requiert des voies de régulation permettant le contrôle de l'expression des gènes impliqués dans la production de matrice ou la motilité. En effet, l'une des caractéristiques des biofilms comme nous l'avons vu précédemment (Synthèse bibliographie : Chapitre 2.2), est leur hétérogénéité cellulaire. Chez *B. subtilis*, la présence de plusieurs sous-populations bactériennes dans le biofilm a été clairement démontrée, suggérant l'existence d'une régulation spatio-temporelle de l'expression génique dans de telles structures 3D (Vlamakis et al., 2008, 2013). Cette hétérogénéité cellulaire est illustrée par la coexistence de différents types cellulaires comme les cellules productrices de matrice, productrices de surfactine, les cellules mobiles flagellées ou encore les cellules sporulées. Chaque type cellulaire est caractérisé par l'expression différentielle de gènes spécifiques régulés par des déterminants génétiques impliqués dans différentes voies de régulation de la formation de biofilms (Fig. 11) (Vlamakis et al., 2008; Mielich-Süss and Lopez, 2015; van Gestel et al., 2015). L'activation de ces différentes voies de régulation passe principalement par la reconnaissance de signaux extracellulaires (présence de biocides, surfactants, exsudats végétaux…) orchestrée par les kinases-phosphatases KinA, KinB, KinC et KinD permettant la phosphorylation du régulateur Spo0A et ainsi activant la formation de biofilm (Vlamakis et al., 2013).

En effet, le régulateur transcriptionnel Spo0A contrôle l'expression d'une centaine de gènes comme par exemple les gènes codant les régulateurs transcriptionnels AbrB, SinI/SinR, SlrA/SlrR, impliqués dans la régulation directe ou indirecte des gènes de production de composants de la matrice extracellulaire (opéron *tasA*, opéron *eps*) ou de motilité (*hag*) (Vlamakis et al., 2013). L'opéron *tasA*, permet la synthèse de la protéine structurale TasA présente dans la matrice et formant des fibres amyloïdes à la surface des cellules. L'opéron *eps* est impliqué dans la production d'exopolysaccharides présents dans la matrice (Branda et al., 2006; Romero et al., 2010; Vlamakis et al., 2013). Le gène *hag* code une protéine flagellaire exprimée dans les cellules motiles (Vlamakis et al., 2013). Spo0A intervient dans la régulation des gènes *srf* permettant la synthèse de surfactine, un lipopeptide tensio-actif impliqué dans la formation de biofilms par induction de la synthèse de polymères et impliqué dans la régulation des gènes *swr* du système de motilité « swarming », caractérisé par un déplacement coordonné des cellules permettant la colonisation d'un milieu (Kearns et al., 2004). Chez les bacilles, la surfactine, en plus de ses propriétés antimicrobiennes, peut agir comme une molécule de signalisation et favoriser la formation de biofilms d'autres bacilles (López et al., 2009; Aleti et al., 2016). Spo0A permet également la régulation du gène *sigF* impliqué dans la sporulation (Vlamakis et al., 2013; Mielich-Süss and Lopez, 2015; van Gestel et al., 2015).

Un autre régulateur, DegU, est également impliqué dans la régulation des gènes de la matrice comme *blsA* codant une hydrophobine (BlsA) formant une couche protectrice hydrophobe à la surface du biofilm limitant la pénétration externe de molécules, ou encore l'opéron *pgsBCD* permettant la synthèse d'un polymère donnant un aspect muqueux aux colonies. DegU est également impliqués dans

la motilité, il contrôle l'expression des gènes *swr* impliqués dans le swarming (Kearns et al., 2004; Verhamme et al., 2007; Vlamakis et al., 2013; Xu et al., 2014; Mielich-Süss and Lopez, 2015). Enfin, le régulateur ComA contrôle également la production de surfactine, ainsi que la capacité d'incorporer l'ADN exogène de l'environnement devenant une nouvelle source de nutriments en contrôlant les gènes *com* (López and Kolter, 2010; Mielich-Süss and Lopez, 2015; van Gestel et al., 2015).

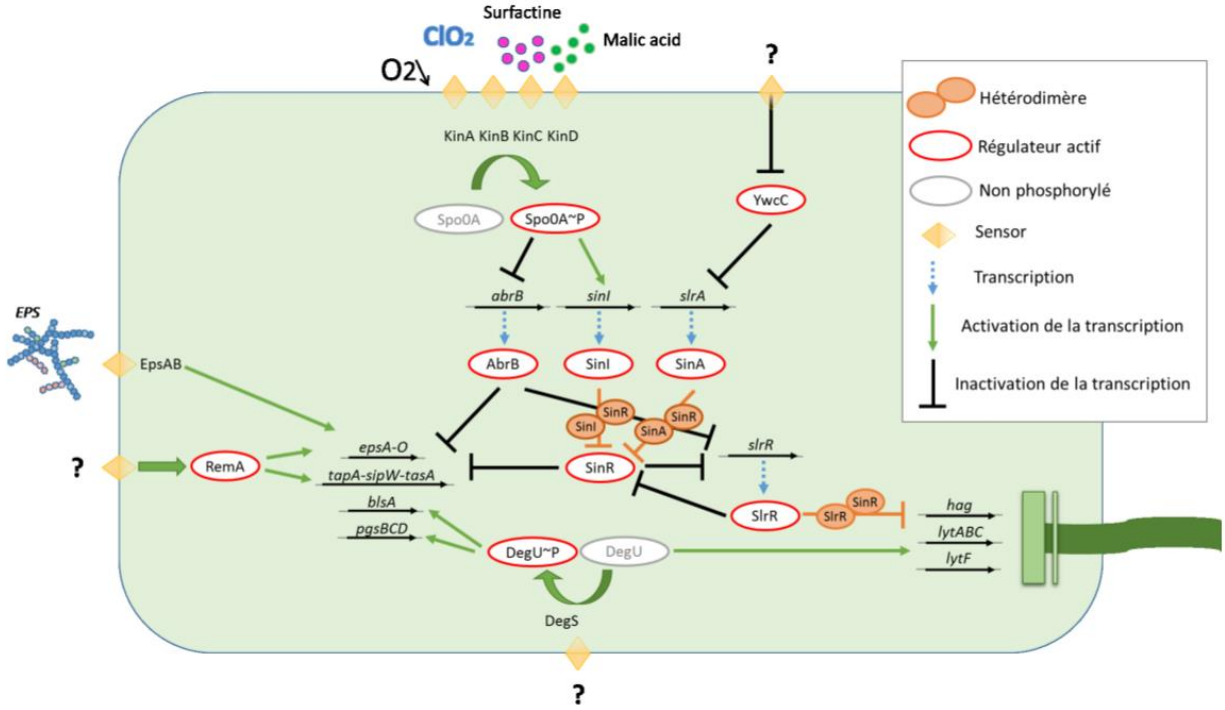

Fig. 11: Réseau de régulation de la formation de biofilms chez B. subtilis (Thèse P. Sanchez-Vizuete, 2015).

Comme nous l'avons vu précédemment au cours du (Synthèse bibliographie : Chapitre 2.2), il a également été montré que certains antimicrobiens pouvait être surproduits en biofilm, conférant de ce fait, un avantage supplémentaire à la protection mise en place par la présence du biofilm. En partant de l'hypothèse que la formation de biofilm par *B. subtilis* QST713 serait impliquée dans les mécanismes de bioprotection s'exerçant contre *T. aggressivum* lors de la culture du champignon de couche, nous nous sommes intéressés à la présence des gènes impliqués dans la formation de biofilm et la production d'antimicrobiens dans le génome de *B. subtilis* QST713. Nous avons donc entrepris de séquencer le génome de la souche QST713, absent des banques de données génomiques, ce qui a permis d'une part de la projeter dans la nouvelle classification taxonomique du « *Bacillus subtilis* species complex », et d'autre part de rechercher la présence de ces gènes dans son génome et de les comparer à d'autres souches de biocontrôle. Nous avons également exploré la capacité de la souche QST713 à former des biofilms et à inhiber le pathogène *T. aggressivum in vitro.* Les résultats de ce chapitre sont présentés sous forme d'un article paru dans *Journal of Biotechnology*, 2018 ; 278:10-19 : « Complete genome sequence of *Bacillus velezensis* QST713: A biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease. ».

1.1 Article 3: "Complete genome sequence of *Bacillus velezensis* QST713: a biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease"

Caroline Pandin[a], Dominique Le Coq[a,b], Julien Deschamps[a], Régis Védie[c], Thierry Rousseau[c], Stéphane Aymerich[a], Romain Briandet[a]

[a] Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[b] Micalis Institute, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[c] Centre Technique du Champignon, 49400 Distré, France.

Journal of Biotechnology, 2018

(http://dx.doi.org/10.1016/j.jbiotec.2018.04.014)

Complete genome sequence of *Bacillus velezensis* QST713: A biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease.

Caroline Pandin[a,*], Dominique Le Coq[a,b], Julien Deschamps[a], Régis Védie[c], Thierry Rousseau[c], Stéphane Aymerich[a], Romain Briandet[a]

[a]Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[b]Micalis Institute, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[c]Centre Technique du Champignon, 49400 Distré, France.

*Corresponding author.
E-mail addresses: caroline.pandin@inra.fr (C. Pandin), romain.briandet@inra.fr (R. Briandet)

ARTICLE INFO

Bacillus velezensis
Biofilm
Genome
Biocontrol
Secondary metabolites

ABSTRACT

Bacillus subtilis QST713 is extensively used as a biological control agent in agricultural fields including in the button mushroom culture, *Agaricus bisporus*. This last use exploits its inhibitory activity against microbial pathogens such as *Trichoderma aggressivum* f. *europaeum*, the main button mushroom green mould competitor. Here, we report the complete genome sequence of this bacterium with a genome size of 4 233 757 bp, 4263 predicted genes and an average GC content of 45.9%. Based on phylogenomic analyses, strain QST713 is finally designated as *Bacillus velezensis*. Genomic analyses revealed two clusters encoding potential new antimicrobials with NRPS and TransATPKS synthetase. *B. velezensis* QST713 genome also harbours several genes previously described as being involved in surface colonization and biofilm formation. This strain shows a strong ability to form *in vitro* spatially organized biofilm and to antagonize *T. aggressivum*. The availability of this genome sequence could bring new elements to understand the interactions with micro or/and macroorganisms in crops.

1 Introduction

Bacillus spp. are ubiquitous bacteria found in soils that are widely used as biocontrol agents because of their ability to antagonize crops pathogens and the possibility of developing a stable spore-based commercial product (Borriss 2015). The described mechanisms associated with their biocontrol effect include specific antagonism, spatial and nutritional competition, antimicrobial production, stimulation of plant growth, and induction of plant resistance (Pandin *et al*. 2017). Bacilli biocontrol agents are able to secrete a wide range of secondary metabolites that can display antibacterial, antifungal, or both properties (Stein 2005; Ongena & Jacques 2008; Chen *et al.* 2009; Zhao & Kuipers 2016). Another ability that should be emphasized in the field of

biocontrol is the formation of spatially organized biofilms, which facilitates better colonization and persistence of microorganisms in environments (Costerton *et al.* 1978, 1999; Morris *et al.* 2003; Epstein *et al.* 2011; Xu *et al.* 2014; Zeriouh *et al.* 2014; Abd el Daim *et al.* 2015; Pandin *et al.* 2017). For almost ten years, *Bacillus velezensis* QST713 has been used for the bioprotection of the button mushroom cultures in France against *Trichoderma aggressivum* f. *europaeum.* This fungal pathogen hinders the development of *Agaricus bisporus* by invading its culture substrate, the compost (Mamoun *et al.* 2000; Samuels *et al.* 2002; Largeteau & Savoie 2010). However, published reports on this specific strain are very scarce and its genome sequence is unknown from databanks (Joshi and McSpadden Gardener 2006; Patel *et al.* 2011; Lahlali *et al.* 2013; Cawoy *et al.* 2015; Punja *et al.* 2016). In this study, the genome sequence of the commercial strain QST713 was determined, described and compared to other *Bacillus* strains. Furthermore, phenotypic tests and biocontrol assay were carried out to emphasis abilities of this biocontrol strain.

Table 1

Comparative genomic analysis of *Bacillus velezensis* QST713 with *Bacillus* genomes.

Strains	Genbank accession N°	dDDH % (± C.I.)	ANI (%)	AAI (%)	GC (%)	bp	Source
Bacillus velezensis							
QST713	**CP025079**	**100 ± 0.0**	**100**	**100**	**45.9**	**4233757**	**Commercial strain used in agriculture (Serenade ® : Bayer, SERENE100/40)**
CFSAN034339	NZ_LYNB00000000	99.5 ± 0.3	99.96	99.89	45.9	4209526	Agricultural soil
Bs006	NZ_LJAU00000000	96.4 ± 1.3	99.97	99.88	45.8	4173094	Banana roots
UMAF6639	NZ_CP006058	92.0 ± 2.1	98.93	99.24	46.3	4034636	Unknown
UCMB5036	NC_020410	92.7 ± 2.0	98.74	99.13	46.6	3910324	Inner tissues of the cotton plant
CC09	NZ_CP015443	91.2 ± 2.2	98.80	98.92	46.1	4167153	Camphor leaves
AS43 3	NC_019842	88.1 ± 2.5	98.37	98.87	46.6	3961368	Surface of a wheat spike
UCMB5113	NC_022081	88.9 ± 2.4	98.23	98.82	46.7	3889532	Soil from Karpaty mountains
TrigoCor1448	NZ_CP007244	88.3 ± 2.4	98.38	98.81	46.5	3957904	Wheat rhizosphere
SB1216	CP015417	88.2 ± 2.5	98.14	98.79	46.8	3814720	Soil
FZB42	NC_009725	88.3 ± 2.5	98.11	98.77	46.5	3918589	infested sugar beet
G341	NZ_CP011686	88.4 ± 2.4	98.17	98.54	46.5	4009746	Korean ginseng rhizosphere
UCMB5033	NC_022075	87.8 ± 2.5	97.92	98.53	46.2	4071167	Cotton rhizosphere
JTYP2	NZ_CP020375	83.4 ± 2.8	97.79	98.50	46.5	3929789	Crassulaceae leaf surface
LS69	NZ_CP015911	83.4 ± 2.8	97.81	98.49	46.5	3917759	isolated from the rice field
S3 1	NZ_CP016371	83.4 ± 2.8	97.79	98.49	46.5	3929772	Cucumber rhizosphere
SYBC H47	NZ_CP017747	81.2 ± 2.9	97.42	98.36	46.4	3884433	Honey
D2 2	NZ_CP014990	79.6 ± 2.9	97.32	98.24	46.7	3921833	Unknown
SQR9	NZ_CP006890	82.9 ± 2.8	97.61	98.11	46.1	4117023	Cucumber rhizosphere
Y2	NC_017912	83.6 ± 2.8	97.62	98.09	45.9	4238624	Wheat rhizosphere
YAUB9601 Y2	NC_017061	83.6 ± 2.8	97.62	98.09	45.9	4242767	Wheat rhizosphere
IT 45	NC_020272	79.8 ± 3.0	97.21	98.07	46.6	3928855	Commercial strain used in horticulture
NJN 6	NZ_CP007165	79.3 ± 2.9	97.44	98.06	46.6	4052546	Banana rhizosphere
M75	NZ_CP016395	79.6 ± 2.9	97.27	98.06	46.6	4007450	Cotton waste
CAUB946	NC_016784	78.9 ± 2.9	97.22	97.97	46.5	4019858	Rice rhizosphere
Bacillus amyloliquefaciens							
DSM 7	NC_014551	55.7 ± 2.7	93.29	95.77	46.1	3980199	Soil, fermentation plant
TA208	NC_017188	55.1 ± 2.7	93.17	95.53	45.8	3937511	Lab stock, overproducing guanosine
Bacillus subtilis							
NDmed	NZ_JPVW00000000	20.5 ± 2.4	76.98	79.97	43.7	4059981	Endoscope washer-disinfector
NCIB 3610	NZ_CP020102	20.9 ± 2.4	76.43	81.00	43.5	4215607	Soil
168	NZ_CP010052	20.9 ± 2.4	76.43	81.00	43.5	4215619	Laboratory strain

Note: Average nucleotide identity (ANI) and average amino acid identity (AAI) calculation was performed *via* the EDGAR platform and the Kostas lab web server (Blom *et al.* 2009, 2016; Goris *et al.* 2007; Rodriguez-R & Konstantinidis 2016; Yoon *et al.* 2017). Digital DNA–DNA hybridization (dDDH) calculation was performed through the DSMZ webserver, the genome-to-genome-distance calculator version 2.1 (GGDC) was used for genome-based species delineation (Meier-Kolthoff et al. 2013) with formula two as described in Fan et al., (2017).

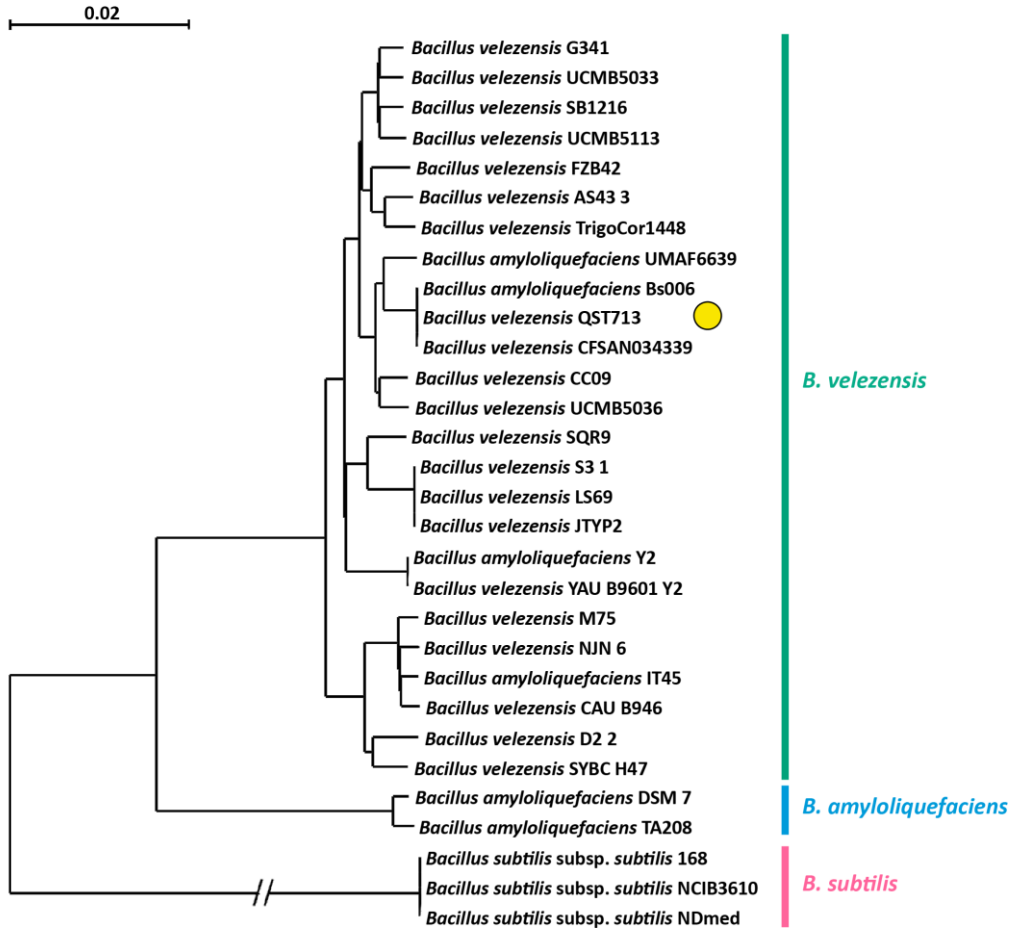

Fig. 1: Neighbor-joining phylogenetic tree constructed from the core genomes of 30 *B. subtilis*, *B. amyloliquefaciens* and *B. velezensis* strains. The position of *Bacillus velezensis* QST713 in the tree is marked by a yellow dot. EDGAR platform was used to obtain alignements in NEXUS format and SeaView 4.5.4 software to construct phylogenetic tree using Neighbor-Joining method (Edgar 2004a,b; Blom *et al*. 2009, 2016; Gouy *et al*. 2009). The bar (0.02) indicates substitutions per nucleotide position.

2 Genomic structure and comparative genome analysis of *Bacillus velezensis* QST713 with *Bacillus* strains

Genomic DNA was extracted from *B. velezensis* QST713 cultivated at 37°C in Luria-Bertani medium (LB, Sigma, France) using the GenElute™ Bacterial Genomic DNA extraction Kit (Sigma-Aldrich, St. Louis) and sequenced using Genome Sequencer Illumina HiSeq2500 technology (GATC Biotech). A total of 6,364,797 high quality filtered paired-end reads of length 126 bp were generated (Bokulich *et al.* 2013). SPAdes was used to assemble the filtered reads (Bankevish *et al.* 2012). Tools used to filter and assemble the reads are available at the Galaxy portal of the MIGALE platform. The obtained contigs were ordered using Mauve genome alignment software v2.1.3 (Darling *et al.* 2004), and assemblies were manually combined into one contig with a total size of 4,23 Mb by identifying overlapping nucleotide sequences in reads and mapping them against genomes of the closest *B. velezensis* strains CC09 (*accession n°:* NZ_CP015443), CFSAN034339 (*accession n°:* LYNB00000000) and Bs006 (*accession n°:* NZ_LJAU00000000). Genome annotation was accomplished using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (Angiuoli *et al.* 2008).

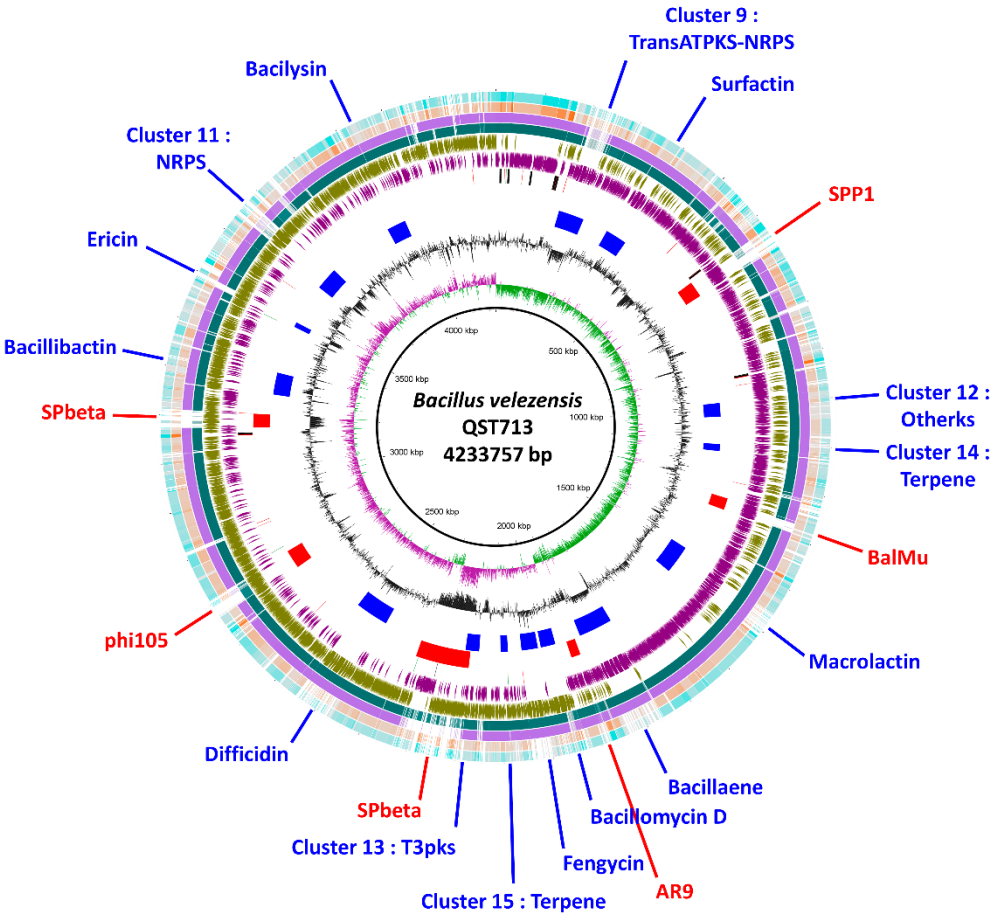

Fig. 2: Circular map of genomic features of the whole genome of *Bacillus velezensis* QST713. Circles display from the inner to outside: (1) GC Skew ; (2) GC Content ; (3) Predictive secondary metabolites clusters ; (4) Predictive prophages clusters; (5) rRNA (black), tRNA (red), ncRNA (green); (6) Predicted CDSs transcribed in clockwise direction; (7) Predicted CDSs transcribed in the counterclockwise direction; (8, 9, 10, 11) Blast comparison of QST713 genome with *Bacillus* strains, SQR9, FZB42, NCIB3610 and 168 respectively. Circular map and blast comparison were performed with BLAST Ring Image Generator software (BRIG) (Alikhan *et al.* 2011).

Table 2: Comparative analysis of secondary metabolites clusters of *Bacillus velezensis* QST713 identified in genome with plant-associated strains and reference genomes.

Bacillus velezensis QST713						Presence (+) or absence (-) of secondary metabolites clusters in *Bacillus* strains			
Cluster number	Synthetase	Metabolites	MIBiG ID (% of genes show similarity)	Predicted large cluster position	bp	FZB42	SQR9	3610	168
1	NRPS	Surfactin	BGC0000433_c1 (86%)	347609 - 413022	65414	+	+	+	+
2	TransATPKS	Macrolactin	BGC0000181_c1 (100%)	1440778 - 1526670	85893	+	+	-	-
3	TransATPKS-NRPS	Bacillaene	BGC0001089_c1 (100%)	1756070 - 1858741	102672	+	+	+	+
4	NRPS	Bacillomycin D	BGC0001090 (66%)	1936517 - 1981185	44669	+	+	-	-
5	TransATPKS-NRPS	Fengycin	BGC0001095_c1 (100%)	1989975 - 2039517	49543	+	+	+	+
6	TransATPKS	Difficidin	BGC0000176_c1 (100%)	2460543 - 2560999	100457	+	+	-	-
7	Bacteriocin-NRPS	Bacillibactin	BGC0001185_c1 (100%)	3272524 - 3339313	66790	+	+	+	+
8	NRPS	Bacilysin	BGC0000309_c1 (100%)	3895380 - 3953631	58252	+	+	+	+
9	TransATPKS-NRPS	Rhizocticin	BGC0000926_c (22%)	191893 - 269628	77736	-	-	-	-
10	Lantipeptide	Subtilin-like / Ericin	BGC0000511_c1 (100%)	3485394 - 3500516	15123	-	-	-	-
11	NRPS	-	-	3625021 - 3693450	68430	-	-	-	-
12	OtherKS	-	-	986643 - 1027887	41245	+	+	-	-
13	T3PKS	-	-	2166999 - 2208099	41101	+	+	+	+
14	Terpene	-	-	1110586 - 1131326	20741	+	+	+	+
15	Terpene	-	-	2080722 - 2102605	21884	+	+	+	+

Note: Prediction of secondary metabolites clusters of *Bacillus* strains were performed through the antiSMASH web server (Medema *et al.* 2011; Blin *et al.* 2013; Weber *et al.* 2015).

Strain QST713 was known as *Bacillus subtilis* QST713 in previous studies (Patel *et al*. 2011; Lahlali *et al*. 2013). However, based on the comparative phylogenomic analysis of *Bacillus* genomes (Table 1; Fig. 1), strain QST713 initially designated as *Bacillus subtilis* should be now reassigned as *Bacillus velezensis* species (formerly known as *Bacillus amyloliquefaciens* subsp. *plantarum*, (Fan *et al*. 2017)). Indeed, digital DNA-DNA Hybridization (dDDH), average nucleotide or amino acid identity (ANI/AAI) analyses showed a higher similarity of strain QST713 with *Bacillus velezensis* species than other *Bacillus* species (Table 1). The genomic structure of the *B. velezensis* QST713 chromosome of 4,233,757 bp length and a Blast comparison with *B. subtilis* and *B. velezensis* strains are represented in Fig. 2. The genome sequence has a G+C content of 45,9% and 4263 predicted genes, including 4056 coding sequences (CDS), 98 pseudo genes, 25 rRNA , 79 tRNA and 5 ncRNA genes. The classification of QST713 genes into clusters of orthologous groups (COG) assigned 2909 CDS in at least one COG group (71.7%) (Fig. S1) (Wu *et al.*, 2011). 1147 CDS were not classified into COG. In those 1147 CDS, 602 were assigned to hypothetical proteins and 545 were conserved proteins, most are parts of prophage regions or involved in sporulation and putative secondary metabolites synthesis. 21% of all assigned COG CDS was dedicated to the transport and metabolism of amino acids, carbohydrates, lipids, inorganic ions, the defence mechanisms and the biosynthesis, transport and catabolism of secondary metabolites. These functions are essentials for a biocontrol agent in terms of nutritional/spatial competition and antagonism against microorganisms to colonize and compete in various ecosystems. PHASTER analysis of *B. velezensis* QST713 genome identified six prophage regions (Table S1) depicted on the red ring in Fig. 2, which represent 8% of the entire genome (Zhou *et al*., 2011; Arndt *et al*., 2016). These regions correspond to variable regions which can be visualized in Fig. 2 (rings 8 - 11) when comparing the genome of *B. velezensis* QST713 with other *Bacillus* genomes, indicating a strong genomic plasticity of strain QST713.

3 Secondary metabolite clusters

Through the antiSMASH genome analysis tool, fifteen clusters of secondary metabolites have been identified in the genome of strain QST713 (Table 2; Fig. 2), five encoding NRPS (Non-Ribosomal Peptide Synthetase), two transATPKS (trans-Acyl Transferase Polyketide Synthetase), three transATPKS-NRPS, two terpene, one lantipeptide, one T3PKS and one otherKS. Nine clusters have been clearly identified as being involved in the synthesis of surfactin, macrolactin, bacillaene, bacillomycin D, fengycin, difficidin, bacilysin, subtilin-like/ericin and bacillibactin (siderophore). All have antimicrobial/antibacterial activities or are implicated in antibiosis (Stein *et al*. 2002, 2005; Ongena *et al*. 2005; Ongena and Jacques 2008; Chen *et al.* 2009; Niazi *et al.* 2014). This analysis revealed that at least 12% of the QST713 genome is allocated to the biosynthesis, regulation and transport of antimicrobials. Secondary metabolites clusters have been compared to four *Bacillus* strains (FZB42, SQR9, NCIB3610 and 168) (Table 2). For all fifteen clusters, genes have been verified, detailed and compared to the reference strain *B. velezensis* FZB42, or when genes were absent from FZB42 strain, to *B. velezensis* CC09 and *Bacillus subtilis* A13 (Table S2). The arrangement of gene clusters in *B. velezensis* QST713 is very similar to those in FZB42 (98-99% nucleotide identity) (Fig. 3; Table S2). *Bacillus velezensis* QST713 has three clusters that are not present in all four *Bacillus* strains (Table 2; Fig. 3). Cluster ten, responsible for the synthesis of subtilin-like/ericin (Genbank accession number **AF233755.1**) and not present in FZB42, is found in *Bacillus subtilis* A13 (unpublished genome) (Stein *et al*. 2002; 2005). We found two clusters encoding potential novel metabolites and not yet described that are similar to gene clusters in CC09 (Fig. 3; Table 2; Table S2). Cluster nine, encoding TransATPKS-NRPS shows 22% of gene similarity with rhizocticin,

but the biosynthetic genes of strain QST713 in this cluster do not correspond to rhizocticin biosynthetic genes from the MIBiG database. In the same way, cluster eleven, encoding NRPS is also unknown from the MIBiG database (Fig. 3; Table 2; Table S2). In accordance with the Cai *et al.* 2017 study, these two clusters could encode two new metabolites specific to *B. velezensis* species.

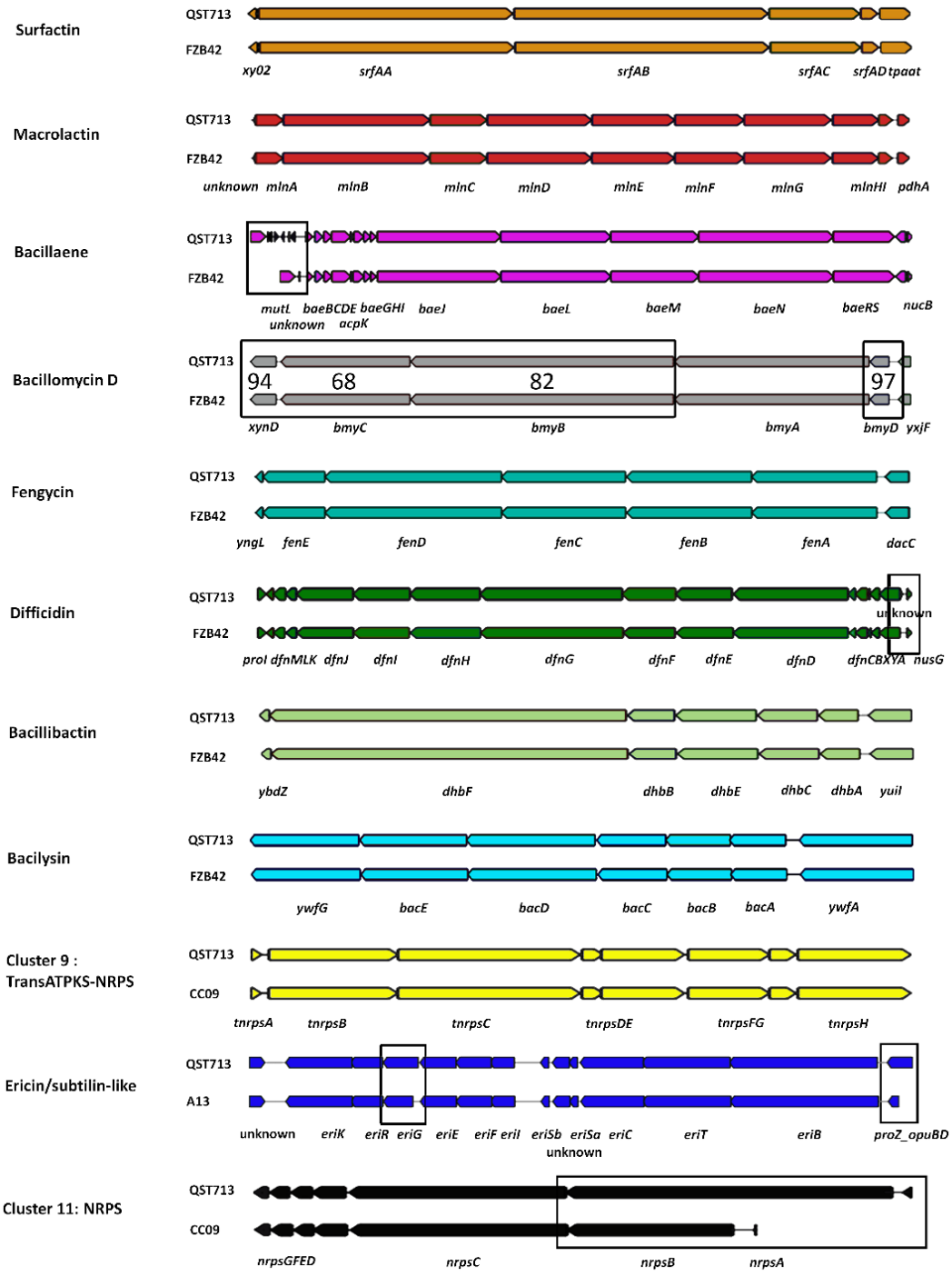

Fig. 3: Comparisons of NRPS, TransATPKS and lantipeptide clusters in QST713 (above), FZB42, A13 or CC09 (below). Squares indicate the differences between strains sequences. Numbers represent the Blast identity percentage between sequences with a coverage of 100% and identity lesser than 98%.

4 Biofilm phenotypes and swarming motility

B. velezensis QST713 is provided with a large number of genes involved in the biofilm formation process (Table 3). Indeed, it has two operons involved in the synthesis of extracellular polysaccharides, *epsA-O* operon and *capABC* (*pgsABC*) operon. It also has the *tasA-sipW-tapA* operon that codes for the TasA protein forming amyloid fibres (Table 3) (de Jong *et al*. 2009; Romero *et al*. 2010). These genes are regulated by a large number of regulatory genes such as *spo0A*, *remA*, *sinI*/*sinR*, *sigW*, *abrB* described in *B. subtilis* and also present in *B. velezensis* QST713 (Winkelman *et al*. 2009; Vlamakis *et al*. 2013).

Table 3: Comparative analysis of genes involved in biofilm formation in *B. velezensis* QST713 and *B. velezensis* FZB42. (*) represents the longest nucleotide sequence taken as a basis for BLAST comparison.

Bacillus velezensis QST713					*B. velezensis* FZB42 Genbank accession n°	
Genes	Product	Accession n°	Position	bp	Coverage / Identity with QST713	
epsO	Operon for capsular biosynthesis	BVQ_17985	3557349 - 3558314	966	RBAM_RS15740	100% / 98%
epsN		BVQ_17990	3558293 - 3559465	1173	RBAM_RS15745	100% / 99%
epsM		BVQ_17995	3559470 - 3560117	648	RBAM_RS15750	100% / 99%
epsL		BVQ_18000	3560114 - 3560722	609	RBAM_RS15755	100% / 99%
epsK		BVQ_18005	3560719 - 3562236	1518	RBAM_RS15760	100% / 99%
epsJ		BVQ_18010	3562233 - 3563267	1035	RBAM_RS15765	100% / 98%
epsI		BVQ_18015	3563264 - 3564340	1077	RBAM_RS15770	100% / 98%
epsH		BVQ_18020	3564345 - 3565382	1038	RBAM_RS15775	100% / 99%
epsG		BVQ_18025	3565401 - 3566504	1104	RBAM_RS15780	100% / 99%
epsF		BVQ_18030	3566508 - 3567644	1137	RBAM_RS15785	100% / 99%
epsE		BVQ_18035	3567637 - 3568479	843	RBAM_RS15790	100% / 99%
epsD		BVQ_18040	3568476 - 3569615	1140	RBAM_RS15795	100% / 98%
epsC		BVQ_18045	3569631 - 3571424	1794	RBAM_RS15800	100% / 99%
epsB		BVQ_18050	3572351 - 3571671	681	RBAM_RS15805	100% / 99%
epsA		BVQ_18055	3573064 - 3572357	708	RBAM_RS15810	100% / 99%
comA	Response regulator in the two-component regulatory system ComP/ComA involved in a major quorum response pathway that regulates the development of genetic competence	BVQ_16475	3266368 - 3265724	645	RBAM_RS14365	100% / 99%
pgcA (*yhxB*)	Operon for poly-γ-glutamate synthesis	BVQ_05065	982326 - 984068	1743	RBAM_RS04780	100% / 99%
pgsA (*capA*)		BVQ_18760	3749291 - 3748143	1149	RBAM_RS16480	100% / 99%
pgsB (*capB*)		BVQ_18770	3750955 - 3749774	1182	RBAM_RS16490	100% / 99%
pgsC (*capC*)		BVQ_18765	3749759 - 3749310	450	RBAM_RS16485	100% / 99%
sinI	SinR antagonist	BVQ_12985	2626360 - 2626533	174	RBAM_RS11410	100% / 99%
sinR	Regulator of biofilm formation	BVQ_12990	2626567 - 2626902	336	RBAM_RS11415*	98% / 99%
tasA	Operon for amyloid fibers biosynthesis and essential for	BVQ_12995	2627735 - 2626950	786	RBAM_RS11420	100% / 99%
sipW	biofilm formation	BVQ_13000	2628384 - 2627800	585	RBAM_RS11425	100% / 99%
tapA (*yqxM*)		BVQ_13005	2629027 - 2628356	672	RBAM_RS11430	100% / 99%
blsA (*yuaB*)	major amphiphilic protein that mediates adherence and forms a highly hydrophobic coat around biofilms	BVQ_15970	3168030 - 3168569	540	RBAM_RS14060	100% / 99%
spo0A	Regulator of initiation of sporulation	BVQ_12800	2593194 - 2592394	801	RBAM_RS11225	100% / 99%
degU	Two-component response regulator	BVQ_18575	3701900 - 3701211	690	RBAM_RS16295	100% / 99%
degQ	Degradation enzyme regulation protein	BVQ_18580	3701977 - 3703140	1164	RBAM_RS16300	100% / 99%
kinA	Kinases involved in multicomponent phosphorelay system	BVQ_07360*	1407114 - 1408934	1821	RBAM_RS06875	100% / 99%
kinB		BVQ_16365	3244655 - 3245938	1284	RBAM_RS14255	100% / 99%
kinC		BVQ_07600	1448464 - 1449768	1305	RBAM_RS07105	100% / 99%
kinD		BVQ_07170*	1370427 - 1368910	1518	RBAM_RS06695	98% / 99%
abrB	Transition state regulator	BVQ_00230	45895 - 45611	285	RBAM_RS00230	100% / 99%
slrA	Transcriptional regulator	BVQ_19965	3965724 - 3965885	162	RBAM_RS17715	100% / 100%
slrR	Transcriptional regulator	BVQ_18060	3573308 - 3573763	456	RBAM_RS15815	99% / 99%
remA	activation of the matrix biosynthesis operons	BVQ_08245	1620674 - 1620943	270	RBAM_RS07740	100% / 100%
lytS (*ypdA*)	Sensor histidine kinase	BVQ_14910	2974431 - 2972650	1782	RBAM_RS12940	100% / 99%
srfAA	Surfactin synthetases	BVQ_01790	367609 - 378366	10758	RBAM_RS01840	100% / 99%
srfAB		BVQ_01795	378388 - 389151	10764	RBAM_RS01845	100% / 99%
srfAC		BVQ_01800	389186 - 393022	3837	RBAM_RS01850	100% / 98%
srfAD		BVQ_01805	393042 - 393773	732	RBAM_RS01855	100% / 99%
sfp	Phosphopantetheinyl transferase necessary for surfactin synthesis	BVQ_01815	395915 - 395241	675	RBAM_RS01880	99% / 99%
swrA	Swarming protein	BVQ_18440	3677165 - 3676830	336	RBAM_RS16160	100% / 95%
swrB	Swarming protein	BVQ_08655	1696313 - 1696780	468	RBAM_RS08150	100% / 99%
swrC	Multidrug efflux pump	BVQ_03840	744422 - 747565	3144	RBAM_RS03555	100% / 99%
sigH	Sigma factor H	BVQ_00560	111395 - 112051	657	RBAM_RS00625	100% / 99%
sigW	ECF sigma factor W	BVQ_01060	188349 - 188912	564	RBAM_RS01130	100% / 99%
motA	Flagellar motor rotation protein	BVQ_07185	1372681 - 1371866	816	RBAM_RS06710	100% / 99%
motB	Flagellar motor rotation protein	BVQ_07180	1371894 - 1371109	786	RBAM_RS06705	100% / 99%
fla-che	Operon for flagellar synthesis and chemotaxis	BVQ_08500 - BVQ_08655	1670445 - 1696780	26335	RBAM_RS07995 - RBAM_RS08150	-100% / 99%
codY	Transcription regulator	BVQ_08495	1669312 - 1670091	780	RBAM_RS07990	100% / 99%
ecsA	ABC transporter ATP-binding protein	BVQ_05445	1050844 - 1051587	744	RBAM_RS05140	100% / 99%
ecsB	ABC transporter permease	BVQ_05450	1051580 - 1052806	1227	RBAM_RS05145	100% / 99%
ecsC	ABC transporter-associated protein	BVQ_05455	1052849 - 1053553	705	RBAM_RS05150	100% / 99%
yusV	Protein involved in swarming/biofilm formation	BVQ_17175	3397437 - 3396613	825	RBAM_RS15000	100% / 99%

Indeed, strain QST713 shows a complex macrocolony structure which is mucoid under a thick and dry surface layer as compared to *B. subtilis* 168 which has flat and non-mucoid macrocolony (Fig. 4). *B. velezensis* SQR9 also shows a complex macrocolony structure but without mucoid aspect. However, *B. velezensis* FZB42 which also harbours these matrix genes shows a flat macrocolony. We obtained similar results for pellicles experiment with a thick and solid pellicle for strain QST713 compared to other strains (Fig. 4). The biofilm experiments show, like macrocolonies and pellicles experiments, more structured and denser cellular aggregates for strain QST713 than for other strains capable of forming biofilms (Fig. 4).

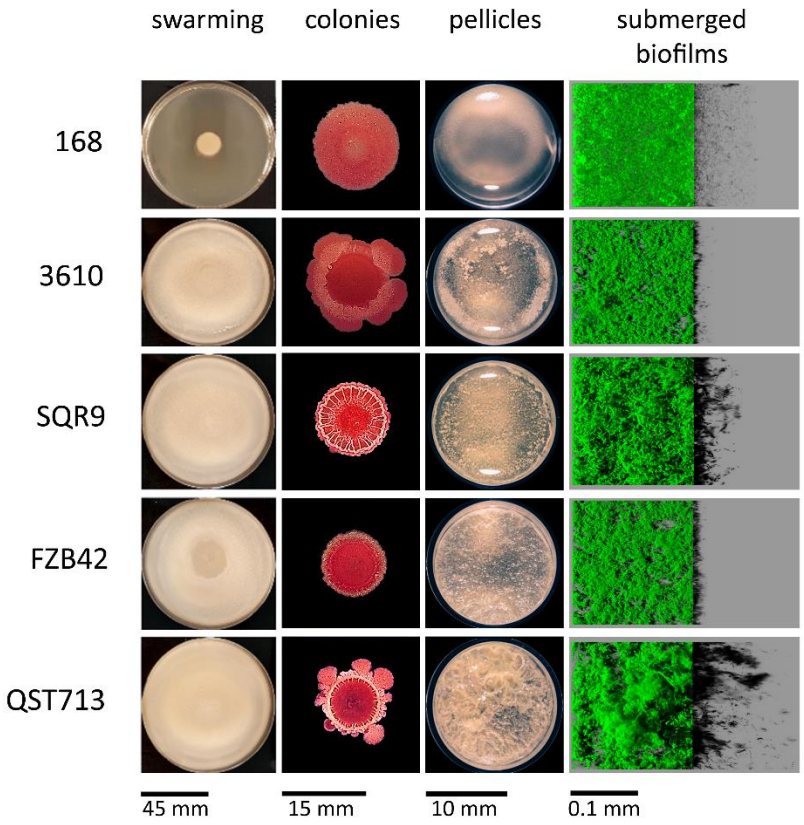

Fig. 4: Phenotypic comparisons of *B. velezensis* QST713 with *Bacillus* species. Strains are *B. subtilis* 168, *B. subtilis* NCIB3610, *B. velezensis* SQR9, *B. velezensis* FZB42 and *B. velezensis* QST713. Biofilm formation was evaluated as macrocolonies morphology, pellicles formation and submerged biofilms. To visualize colony architecture, 5 µl of an overnight culture in TSB were spotted on 1.5% Tryptone Soya Agar (TSA, Biomérieux, France) with 40 µg ml^{-1} of Congo red to visualize matrix elements (Jones and Wozniak 2017). For pellicles visualisation, 10 µl of an overnight culture in TSB were used to inoculate 2 ml of TSB in 24-well plates (TPP, Switzerland). Plates were then incubated at 25°C for 72h, macrocolonies and pellicles formation were recorded at 72h and digital pictures were taken. Submerged biofilm formation was performed in 96-well microtiter plates (Greiner µClear®, Germany). Briefly, for each strain, 200 µl of an overnight culture in TSB standardized to an OD_{600nm} of 0.01 were added to the wells, then incubated at 25°C for 90 min for attachment. The supernatant was removed to eliminate non-adherent bacteria and 200 µl of sterile TSB was added. Plates were then incubated for 24h at 25°C, experiment was repeated six times. SYTO9® (Invitrogen, France), a green-fluorescent nucleic acid marker was used to tag the cells fluorescently for submerged-biofilm observations by confocal laser scanning microscopy (Leica SP8, Leica Mycrosystems, Germany). Swarming motility of *Bacillus* strains was determined by the spread of bacteria on TSA 0.8% agar plates at 25° for 24h, 10 µl of an overnight culture were inoculated at the centre of the 9 cm agar plates dried for 15 min and incubated at 25°C for 24h. Experiments was repeated three times.

As observed by Magno-Pérez-Bryan *et al.* (2015) and Norman *et al*. (2013), our results suggest that other regulatory pathways or epigenetic events might be involved in biofilm formation and related phenotype expressions.

Swarming motility is an essential skill to colonize environmental surfaces such as soils or crops tissues (Fan *et al.* 2011; Mordini *et al*. 2013). *B. velezensis* QST713 possesses the *swr* genes necessary for swarming motility but also the *fla-che* operon including *fla* genes that code for flagellar components and are up-regulated by *swrA*, thus improving swarming motility (Kearns *et al*. 2004; Ghelardi *et al*. 2012). All the strains tested except the negative swarming control *B. subtilis* 168 showed a swarming motility characterized by the invasion of 9 cm diameters of TSA 0.8% agar plates at 25°C in 24 h (Fig. 4). Efficient swarming and biofilm formation confer to biocontrol agents a better fitness to colonize substrata and to outcompete pathogens.

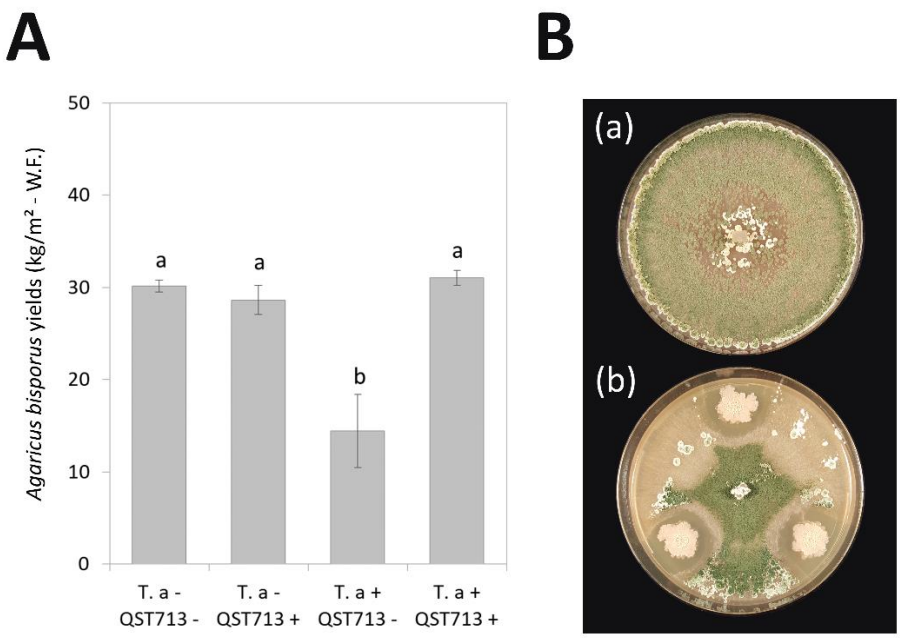

Fig. 5: Biocontrol effect of *Bacillus velezensis* QST713 against *T. aggressivum* f. *europaeum*. (A) Button mushroom yields of *A. bisporus* when the culture compost is contaminated or not by *T. aggressivum* (T. a) and treated or not by strain QST713: (+) Presence of; (-) Absence of. *Agaricus bisporus* was seeded at a rate of 0,8% of compost weight (Amycel Delta, white hybrid variety, Amycel, Vendôme). Treatment with *B. velezensis* QST713: compost was homogenized with suspension of *B. velezensis* QST713 at a rate of 0.1 g kg^{-1} of product (Serenade Max ®, AMM No. 2100162, BayerCropScience, France) corresponding at 5.10^6 CFU gr^{-1} of compost. Inoculation with *T. aggressivum* with 2000 spores in a central spot in pot of 9 kg of compost. Yield data correspond at the number in kg per square meter of button mushroom whole feets collected form a complete culture cycle of 2 months. Experiment was repeated 6 times. (B) Antagonism test of *T. aggressivum* by *B. velezensis* QST713: (a) *T. aggressivum* alone; (b) *T. aggressivum vs B. velezensis* QST713. The fungal pathogen was inoculated with an agar plug taken from a 72 h fungal culture that was deposited on the center of YMEA medium plate (yeast extract 2 g L^{-1}, malt extract 20 g L^{-1}, agar 15 g L^{-1}). 5 µl of sterile water (control) or of an overnight culture of *B. velezensis* QST713 in TSB was inoculated in three spots at 3 cm of *T. aggressivum* spot. Petri dish were incubated at 25°C and observations were performed 5 days after by measuring the inhibition zone of *T. aggressivum*. Experiment was repeated 3 times. Differences among treatments were determined by performing a one-way analysis of variance ($P < 0.05$) and Tukey's honestly significant difference (HSD) procedure ($P < 0.05$) with Statgraphics Centurion software (Statpoint Technologies, United States). Letters a and b on the graph represent significant differences ($P < 0.05$).

5 Biocontrol effect of *B. velezensis* QST713 against the green mould *T. aggressivum*

Biocontrol assay with *B. velezensis* QST713 in the mushroom field and antagonism tests by measuring inhibition zones surrounding *Bacillus* strains were performed (Fig. 5). Biocontrol assay was carried out from November 2017 to January 2018 at the technical mushroom center (CTC, Distré, France). Results show a strong effect of strain QST713 on the yield of *A. bisporus* crops infected by *T. aggressivum*. When the culture compost is contaminated by *T. aggressivum*, we observed a diminution of the yield of *A. bisporus* of about 50% (Fig. 5A). However, when the compost contaminated is treated by strain QST713, the yield of *A. bisporus* returned to a value similar to the control yield (Fig. 5A). Furthermore, antagonism tests on *B. velezensis* QST713 showed a strong efficacy against the green mould by a direct inhibition (inhibition zone: 4.7 ± 0.5 mm) of the growth of mycelium and an inhibition of fungus sporulation (diminution of green colour) compared to *T. aggressivum* alone (Fig. 5B(a),(b)). This second antagonism effect which is not located at the direct interaction interface, could be due to a secretion of volatile organic compounds by QST713. This phenomenon has already been demonstrated by strain QST713 on the inhibition of sporulation of *Streptomyces scabies* (Meng *et al*. 2016).

6 Conclusion

The phylogenetic analysis of QST713 genome allows a reassignment of strain QST713 as a *Bacillus velezensis* strain. Its ability to form highly structured, thick and dense biofilms *in vitro* and its ability to swarm on agar plates seemingly suggest its ability to efficiently colonise and persist in crops. In addition, it is equipped with an antimicrobial arsenal enabling it to fight with aggressors, including ericin as well as potentially one NRPS and one transATPKS-NRPS not yet characterized and found only in few strains of *B. velezensis*. All these features allow *B. velezensis* QST713 to be highly competitive in the environment and makes it a particularly efficient biocontrol agent.

Nucleotide sequence accession numbers

The complete genome sequence of *B. velezensis* QST713 has been deposited in NCBI under the GenBank accession number **CP025079**.

Acknowledgements

C. Pandin is granted a doctoral fellowship by the Ile-de-France Region, DIM ASTREA (project n° ast150075). We thank the MIMA2 platform (www6.jouy.inra.fr/mima2) for accessing to the confocal microscope Leica SP8. We thank R. Borriss and R. Zhang for providing the *Bacillus amyloliquefaciens* FZB42 and SQR9 strains, respectively. We are grateful to the EDGAR (https://edgar.computational.bio.uni-giessen.de/) and INRA MIGALE (http://migale.jouy.inra.fr) bioinformatics platforms for providing computational resources.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jbiotec.2018.04.014

References

Abd El Daim, I.A., Häggblom, P., Karlsson, M., Stenström, E., Timmusk, S., 2015. *Paenibacillus polymyxa* A26 Sfp-type PPTase inactivation limits bacterial antagonism against *Fusarium graminearum* but not of *F. culmorum* in kernel assay. Front. Plant Sci. 6, 368. https://doi.org/10.3389/fpls.2015.00368

Alikhan, N.-F., Petty, N.K., Ben Zakour, N.L., Beatson, S.A., 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12, 402. https://doi.org/10.1186/1471-2164-12-402

Angiuoli, S. V., Gussman, A., Klimke, W., Cochrane, G., Field, D., Garrity, G.M., Kodira, C.D., Kyrpides, N., Madupu, R., Markowitz, V., Tatusova, T., Thomson, N., White, O., 2008. Toward an online repository of standard operating procedures (SOPs) for (meta)genomic

annotation. *OMICS*. 12, 137–141. https://doi.org/10.1089/omi.2008.0017

Arndt, D., Grant, J.R., Marcu, A., Sajed, T., Pon, A., Liang, Y., Wishart, D.S., 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res. 44, 16–21. https://doi.org/10.1093/nar/gkw387

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021

Blin, K., Medema, M.H., Kazempour, D., Fischbach, M.A., Breitling, R., Takano, E., Weber, T., 2013. antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res. 41, W204–W212. https://doi.org/10.1093/nar/gkt449

Blom, J., Albaum, S.P., Doppmeier, D., Puhler, A., Vorholter, F.-J., Zakrzewski, M., Goesmann, A., 2009. EDGAR: a software framework for the comparative analysis of prokaryotic genomes. BMC Bioinf 10, 154. https://doi.org/10.1186/1471-2105-10-154

Blom, J., Kreis, J., Spänig, S., Juhre, T., Bertelli, C., Ernst, C., Goesmann, A., 2016. EDGAR 2.0: an enhanced software platform for comparative gene content analyses. Nucleic Acids Res. 44, W22–W28. https://doi.org/10.1093/nar/gkw255

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59. https://doi.org/10.1038/nmeth.2276

Borriss, R., 2015. *Bacillus*, a plant-beneficial bacterium, Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture. Springer International Publishing, Cham, pp. 379–391. https://doi.org/10.1007/978-3-319-08575-3_40

Cai, X.C., Liu, C.H., Wang, B.T., Xue, Y.R., 2017. Genomic and metabolic traits endow *Bacillus velezensis* CC09 with a potential biocontrol agent in control of wheat powdery mildew disease. Microbiol. Res. 196, 89–94. https://doi.org/10.1016/j.micres.2016.12.007

Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., Ongena, M., 2015. Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. Microb. Biotechnol. 8, 281–295. https://doi.org/10.1111/1751-7915.12238

Chen, X.H., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Süssmuth, R., Piel, J., Borriss, R., 2009. Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. J. Biotechnol. 140, 27–37. https://doi.org/10.1016/j.jbiotec.2008.10.011

Costerton, J.W., Stewart, P.S., Greenberg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. Science. 284, 1318-1322. https://doi.org/10.1126/science.284.5418.1318

Costerton, J.W., Geesey, G.G., Cheng, K.J., 1978. How bacteria stick. Sci. Am. 238, 86–95. https://doi.org/10.1038/scientificamerican0178-86

Darling, A.C.E., Mau, B., Blattner, F.R., Perna, N.T., 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res. 14, 1394–1403. https://doi.org/10.1101/gr.2289704

De Jong, W., Wösten, H.A.B., Dijkhuizen, L., Claessen, D., 2009. Attachment of *Streptomyces coelicolor* is mediated by amyloida fimbriae that are anchored to the cell surface via cellulose. Mol. Microbiol. 73, 1128–1140. https://doi.org/10.1111/j.1365-2958.2009.06838.x

Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5, 113. https://doi.org/10.1186/1471-2105-5-113

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340

Epstein, A.K., Pokroy, B., Seminara, A., Aizenberg, J., 2011. Bacterial biofilm shows persistent resistance to liquid wetting and gas penetration. Proc. Natl. Acad. Sci. 108, 995–1000. https://doi.org/10.1073/pnas.1011033108

Fan, B., Blom, J., Klenk, H.P., Borriss, R., 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "operational group *B. amyloliquefaciens*" within the *B. subtilis* species complex. Front. Microbiol. 8, 22. https://doi.org/10.3389/fmicb.2017.00022

Fan, B., Chen, X.H., Budiharjo, A., Bleiss, W., Vater, J., Borriss, R., 2011. Efficient colonization of plant roots by the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42, engineered to express green fluorescent protein. J. Biotechnol. 151, 303–311. https://doi.org/10.1016/j.jbiotec.2010.12.022

Ghelardi, E., Salvetti, S., Ceragioli, M., Gueye, S.A., Celandroni, F., Senesi, S., 2012. Contribution of surfactin and SwrA to flagellin expression, swimming, and surface motility in *Bacillus subtilis*. Appl. Environ. Microbiol. 78, 6540–6544. https://doi.org/10.1128/AEM.01341-12

Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J.M., 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 57, 81–91. https://doi.org/10.1099/ijs.0.64483-0

Gouy, M., Guindon, S., Gascuel, O., 2010. Sea view version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27, 221–224. https://doi.org/10.1093/molbev/msp259

Jones, C.J., Wozniak, D.J., 2017. Congo red stain identifies matrix overproduction and is an indirect measurement for c-di-GMP in many species of bacteria, in: Methods

in Molecular Biology. Humana Press, New York, NY, pp. 147–156. https://doi.org/10.1007/978-1-4939-7240-1_12

Joshi, R., McSpadden Gardener, B.B., 2006. Identification and characterization of novel genetic markers associated with biological control activities in *Bacillus subtilis*. Phytopathology 96, 145–154. https://doi.org/10.1094/PHYTO-96-0145

Kearns, D.B., Chu, F., Rudner, R., Losick, R., 2004. Genes governing swarming in *Bacillus subtilis* and evidence for a phase variation mechanism controlling surface motility. Mol. Microbiol. 52, 357–369. https://doi.org/10.1111/j.1365-2958.2004.03996.x

Lahlali, R., Peng, G., Gossen, B.D., McGregor, L., Yu, F.Q., Hynes, R.K., Hwang, S.F., McDonald, M.R., Boyetchko, S.M., 2013. Evidence that the biofungicide serenade (*Bacillus subtilis*) suppresses clubroot on canola via antibiosis and induced host resistance. Phytopathology 103, 245–254. https://doi.org/10.1094/PHYTO-06-12-0123-R

Largeteau, M.L., Savoie, J.-M., 2010. Microbially induced diseases of *Agaricus bisporus*: biochemical mechanisms and impact on commercial mushroom production. Appl. Microbiol. Biotechnol. 86, 63–73. https://doi.org/10.1007/s00253-010-2445-2

Magno-Pérez-Bryan, M.C., Martínez-García, P.M., Hierrezuelo, J., Rodríguez-Palenzuela, P., Arrebola, E., Ramos, C., de Vicente, A., Pérez-García, A., Romero, D., 2015. Comparative genomics within the *Bacillus* genus reveal the singularities of two robust *Bacillus amyloliquefaciens* biocontrol strains. Mol. Plant-Microbe Interact. 28, 1102–1116. https://doi.org/10.1094/MPMI-02-15-0023-R

Mamoun, M.L., Iapicco, R., Savoie, J.-M., Olivier, J.M., 2011. Green mould disease in France: *Trichoderma harzianum* Th2 and other species causing damage on mushroom farms, Mushroom Sci. 15, 625-632.

Medema, M.H., Blin, K., Cimermancic, P., De Jager, V., Zakrzewski, P., Fischbach, M.A., Weber, T., Takano, E., Breitling, R., 2011. AntiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res. 39, W339–W346. https://doi.org/10.1093/nar/gkr466

Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Göker, M., 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinf. 14, 60. https://doi.org/10.1186/1471-2105-14-60

Meng, Q., Jiang, H., Hao, J.J., 2016. Effects of *Bacillus velezensis* strain BAC03 in promoting plant growth. Biol. Control 98, 18–26. https://doi.org/10.1016/j.biocontrol.2016.03.010

Mordini, S., Osera, C., Marini, S., Scavone, F., Bellazzi, R., Galizzi, A., Calvio, C., 2013. The role of SwrA, DegU and PD3 in *fla/che* expression in *B. subtilis*. PLoS One 8, e85065. https://doi.org/10.1371/journal.pone.0085065

Morris, C.E., Monier, J.-M., 2003. The ecological significance of biofilm formation by plant-associated bacteria. Annu. Rev. Phytopathol. 41, 429–453. https://doi.org/10.1146/annurev.phyto.41.022103.134521

Niazi, A., Manzoor, S., Asari, S., Bejai, S., Meijer, J., Bongcam-Rudloff, E., 2014. Genome analysis of *Bacillus amyloliquefaciens* subsp. *plantarum* UCMB5113: a rhizobacterium that improves plant growth and stress management. PLoS One 9, e104651. https://doi.org/10.1371/journal.pone.0104651

Norman, T.M., Lord, N.D., Paulsson, J., Losick, R., 2013. Memory and modularity in cell-fate decision making. Nature 503, 481–486. https://doi.org/10.1038/nature12804

Ongena, M., Jacques, P., 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 16, 115-125. https://doi.org/10.1016/j.tim.2007.12.009

Ongena, M., Jacques, P., Touré, Y., Destain, J., Jabrane, A., Thonart, P., 2005. Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of *Bacillus subtilis*. Appl. Microbiol. Biotechnol. 69, 29–38. https://doi.org/10.1007/s00253-005-1940-3

Pandin, C., Le Coq, D., Canette, A., Aymerich, S., Briandet, R., 2017. Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? Microb. Biotechnol. 10, 719-734. https://doi.org/10.1111/1751-7915.12693

Patel, H., Tscheka, C., Edwards, K., Karlsson, G., Heerklotz, H., 2011. All-or-none membrane permeabilization by fengycin-type lipopeptides from *Bacillus subtilis* QST713. Biochim. Biophys. Acta - Biomembr. 1808, 2000–2008. https://doi.org/10.1016/j.bbamem.2011.04.008

Punja, Z.K., Rodriguez, G., Tirajoh, A., 2016. Effects of *Bacillus subtilis* strain QST 713 and storage temperatures on post-harvest disease development on greenhouse tomatoes. Crop Prot. 84, 98–104. https://doi.org/10.1016/j.cropro.2016.02.011

Rodriguez-R, L.M., Konstantinidis, K.T., 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. PeerJ Prepr. 4, e1900v1. https://doi.org/10.7287/peerj.preprints.1900v1

Romero, D., Aguilar, C., Losick, R., Kolter, R., 2010. Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. Proc. Natl. Acad. Sci. 107, 2230–2234. https://doi.org/10.1073/pnas.0910560107

Samuels, G.J., Dodd, S.L., Gams, W., Castlebury, L.A., Petrini, O., 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia 94, 146–170. https://doi.org/10.1080/15572536.2003.11833257

Stein, T., 2005. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol. Microbiol. 56,

845-857. https://doi.org/10.1111/j.1365-2958.2005.04587.x

Stein, T., Borchert, S., Conrad, B., Feesche, J., Hofemeister, B., Hofemeister, J., Entian, K.D., 2002. Two different lantibiotic-like peptides originate from the ericin gene cluster of *Bacillus subtilis* A1/3. J. Bacteriol. 184, 1703–1711. https://doi.org/10.1128/JB.184.6.1703-1711.2002

Vlamakis, H., Chai, Y., Beauregard, P., Losick, R., Kolter, R., 2013. Sticking together: building a biofilm the *Bacillus subtilis* way. Nat. Rev. Microbiol. 11, 157-168. https://doi.org/10.1038/nrmicro2960

Weber, T., Blin, K., Duddela, S., Krug, D., Kim, H.U., Bruccoleri, R., Lee, S.Y., Fischbach, M.A., Müller, R., Wohlleben, W., Breitling, R., Takano, E., Medema, M.H., 2015. AntiSMASH 3.0-A comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res. 43, W237–W243. https://doi.org/10.1093/nar/gkv437

Winkelman, J.T., Blair, K.M., Kearns, D.B., 2009. RemA (YlzA) and RemB (YaaB) regulate extracellular matrix operon expression and biofilm formation in *Bacillus subtilis*. J. Bacteriol. 191, 3981–3991. https://doi.org/10.1128/JB.00278-09

Wu, S., Zhu, Z., Fu, L., Niu, B., Li, W., 2011. WebMGA: a customizable web server for fast metagenomic sequence analysis. BMC Genomics 12, 444. https://doi.org/10.1186/1471-2164-12-444

Xu, Z., Zhang, R., Wang, D., Qiu, M., Feng, H., Zhang, N., Shen, Q., 2014. Enhanced control of cucumber wilt disease by *Bacillus amyloliquefaciens* SQR9 by altering the regulation of its DegU phosphorylation. Appl. Environ. Microbiol. 80, 2941–2950. https://doi.org/10.1128/AEM.03943-13

Yoon, S.H., Ha, S. min, Lim, J., Kwon, S., Chun, J., 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 110, 1281–1286. https://doi.org/10.1007/s10482-017-0844-4

Zeriouh, H., de Vicente, A., Pérez-García, A., Romero, D., 2014. Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. Environ. Microbiol. 16, 2196–2211. https://doi.org/10.1111/1462-2920.12271

Zhao, X., Kuipers, O.P., 2016. Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. BMC Genomics 17, 882. https://doi.org/10.1186/s12864-016-3224-y

Zhou, Y., Liang, Y., Lynch, K.H., Dennis, J.J., Wishart, D.S., 2011. PHAST: a fast phage search tool. Nucleic Acids Res. 39, W347–W352. https://doi.org/10.1093/nar/gkr485

Table S1: Prophage regions of *Bacillus velezensis* QST713 genome.

Region	Region length	Completeness	Number of CDS	Phage Hit protein	Hypothetical protein	Specific Keyword	Region_position	Possible phage	G+C percentage
1	44.8Kb	questionable	71	45	26	integrase, terminase, portal, capsid, tail	630065-674933	PHAGE_Bacill_SPP1_NC_004166	43.88%
2	32.8Kb	questionable	45	32	13	terminase, head, capsid, tail, portal, plate	1260401-1293209	PHAGE_Bacill_BalMu_1_NC_030945	46.83%
3	26.4Kb	incomplete	11	8	3	tail, recombinase	1876725-1903221	PHAGE_Bacill_AR9_NC_031039	41.48%
4	150.2Kb	incomplete	180	112	68	virion, tail, recombinase	2192669-2342879	PHAGE_Bacill_SPbeta_NC_001884	36.62%
5	52.5Kb	questionable	53	41	12	integrase, portal	2760236-2812785	PHAGE_Bacill_phi105_NC_004167	43.51%
6	38.5Kb	questionable	30	22	8	tail, head, capsid, portal	3173273-3211799	PHAGE_Bacill_SPbeta_NC_001884	36.03%

Note: Prophage regions were identified using PHASTER web server (Zhou *et al*. 2011; Arndt *et al*. 2016).

Table S2: Comparative analysis of gene clusters encoding secondary metabolites in *B. velezensis* QST713 with *Bacillus* strains. (*) represents the longest nucleotide sequence taken as a basis for BLAST comparison.

***Bacillus velezensis* QST713**							**Genbank accession number Blast Coverage / Identity nt sequence QST713 with *Bacillus species***	
Cluster number	**Metabolites**	**Synthetase**	**Main genes**	**Accession**	**Position**	**bp**	***B. velezensis* FZB42**	
1	**Surfactin**	**NRPS**	*xy02*	BVQ_01785	367201 - 367425	225	RBAM_RS01835	100% / 99%
			srfAA	BVQ_01790	367609 - 378366	10758	RBAM_RS01840	100% / 99%
			srfAB	BVQ_01795	378388 - 389151	10764	RBAM_RS01845	100% / 99%
			srfAC	BVQ_01800	389186 - 393022	3837	RBAM_RS01850	100% / 98%
			srfAD	BVQ_01805	393042 - 393773	732	RBAM_RS01855	100% / 99%
			tpaat	BVQ_01810	393895 - 395205	1311	RBAM_RS01860	100% / 99%
2	**Macrolactin**	**TransATPKS**	unknown	BVQ_07650	1458250 - 1458449	200	RBAM_RS07155	98% / 99%
			mlnA	BVQ_07655	1458450 - 1460756	2307	RBAM_RS07160	100% / 99%
			mlnB	BVQ_07660	1460778 - 1473032	12255	RBAM_RS07165*	100% / 99%
			mlnC	BVQ_07665	1473032 - 1477804	4773	RBAM_RS07170	100% / 98%
			mlnD	BVQ_07670	1477851 - 1486559	8709	RBAM_RS07175	100% / 99%
			mlnE	BVQ_07675	1486552 - 1493556	7005	RBAM_RS07180	100% / 99%
			mlnF	BVQ_07680	1493580 - 1499291	5712	RBAM_RS07185	100% / 99%
			mlnG	BVQ_07685	1499291 - 1506670	7380	RBAM_RS07190	100% / 99%
			mlnH	BVQ_07690	1506721 - 1510569	3849	RBAM_RS07195*	100% / 98%
			mlnI	BVQ_07695	1510602 - 1511693	1092	RBAM_RS07200	100% / 99%
			pdhA	BVQ_07700	1512163 - 1513278	1116	RBAM_RS07205	100% / 99%
3	**Bacillaene**	**TransATPKS-NRPS**	*mutL*	BVQ_08940*	1760813 - 1762687	1875	RBAM_RS08435	99% / 99%
			unknown	BVQ_08945	1762739 - 1762888	150	-	-
			unknown	BVQ_08950	1762848 - 1763354	507	-	-
			unknown	BVQ_08955	1763792 - 1764094	303	-	-
			unknown	BVQ_08960	1764573 - 1764782	210	-	-
			unknown	BVQ_08965*	1765319 - 1765528	210	RBAM_RS08440	99% - 97%
			unknown	BVQ_08970	1765615 - 1766136	522	-	-
			unknown	BVQ_08975	1767460 - 1767675	216	-	-
			baeB	BVQ_08980	1767659 - 1768336	678	RBAM_RS08445	100% / 99%
			baeC	BVQ_08985	1768651 - 1769520	870	RBAM_RS08450	100% / 99%
			baeD	BVQ_08990	1769657 - 1770631	975	RBAM_RS08455	100% / 99%
			baeE	BVQ_08995	1770633 - 1772873	2241	RBAM_RS08460	100% / 99%
			acpK	BVQ_09000	1772939 - 1773187	249	RBAM_RS08465	100% / 99%
			baeG	BVQ_09005	1773239 - 1774501	1263	RBAM_RS08470	100% / 98%
			baeH	BVQ_09010	1774498 - 1775271	774	RBAM_RS08475	100% / 99%
			baeI	BVQ_09015	1775281 - 1776030	750	RBAM_RS08480	100% / 100%
			baeJ	BVQ_09020*	1776070 - 1791021	14952	RBAM_RS08485	100% / 98%
			baeL	BVQ_09025	1791023 - 1804429	13407	RBAM_RS08490*	100% / 98%
			baeM	BVQ_09030	1804447 - 1814982	10536	RBAM_RS08495	100% / 98%
			baeN	BVQ_09035	1814972 - 1831270	16299	RBAM_RS08500*	100% / 98%
			baeR	BVQ_09040*	1831284 - 1838741	7458	RBAM_RS08505	100% / 98%
			baeS	BVQ_09045	1840089 - 1838878	1212	RBAM_RS08510	100% / 99%
			nucB	BVQ_09050	1840378 - 1840812	435	RBAM_RS08515	100% / 99%
4	**Bacillomycin D**	**NRPS**	*xynD*	BVQ_09605	1937867 - 1939405	1539	RBAM_RS09060	99% / 94%
			bmyC	BVQ_09610	1939730 - 1947586	7857	RBAM_RS09065*	100% / 68%
			bmyB	BVQ_09615	1947675 - 1963763	16089	RBAM_RS09070*	100% / 82%
			bmyA	BVQ_09620	1963808 - 1975756	11949	RBAM_RS09075	100% / 99%
			bmyD	BVQ_09625	1975776 - 1976978	1203	RBAM_RS09080	100% / 97%
			yxjF	BVQ_09635	1977536 - 1978321	786	RBAM_RS09085	100% / 98%
5	**Fengycin**	**TransATPKS-NRPS**	*yngL*	BVQ_09740	1999440 - 1999820	381	RBAM_RS09190	100% / 99%
			fenE	BVQ_09745	2003696 - 1999893	3804	RBAM_RS09195	100% / 98%
			fenD	BVQ_09750	2014490 - 2003715	10776	RBAM_RS09200	100% / 99%
			fenC	BVQ_09755	2022165 - 2014516	7650	RBAM_RS09205	100% / 98%
			fenB	BVQ_09760	2029878 - 2022181	7698	RBAM_RS09210	100% / 98%
			fenA	BVQ_09765	2037562 - 2029904	7659	RBAM_RS09215	100% / 98%
			dacC	BVQ_09775	2039517 - 2038042	1476	RBAM_RS09220	100% / 98%
6	**Difficidin**	**TransATPKS**	*proI*	BVQ_12475	2476320 - 2477159	840	RBAM_RS10905	100% / 99%
			dfnM	BVQ_12480	2477942 - 2477196	747	RBAM_RS10910	100% / 98%
			dfnL	BVQ_12485	2479249 - 2478002	1248	RBAM_RS10915	99% / 99%
			dfnK	BVQ_12490	2480461 - 2479307	1155	RBAM_RS10920	100% / 99%
			dfnJ	BVQ_12495	2486758 - 2480543	6216	RBAM_RS10925	100% / 99%
			dfnI	BVQ_12500	2492907 - 2486755	6153	RBAM_RS10930	100% / 99%
			dfnH	BVQ_12505	2500648 - 2492930	7719	RBAM_RS10935	100% / 98%
			dfnG	BVQ_12510	2516267 - 2500653	15615	RBAM_RS10940	100% / 98%
			dfnF	BVQ_12515	2522045 - 2516319	5727	RBAM_RS10945	100% / 98%
			dfnE	BVQ_12520	2528381 - 2522085	6297	RBAM_RS10950	100% / 98%
			dfnD	BVQ_12525*	2540999 - 2528400	12600	RBAM_RS10955	100% / 98%

			dfnC	BVQ_12530	2541776 - 2541039	738	RBAM_RS10960	100% / 99%
			dfnB	BVQ_12535	2543155 - 2541791	1365	RBAM_RS10965	100% / 99%
			dfnX	BVQ_12540	2543424 - 2543152	273	RBAM_RS10970	100% / 99%
			dfnY	BVQ_12545	2544429 - 2543449	981	RBAM_RS10975	100% / 99%
			dfnA	BVQ_12550	2546728 - 2544470	2259	RBAM_RS10980	100% / 99%
			unknown	BVQ_12555	2546772 - 2546957	186	-	-
			nusG	BVQ_12560	2547462 - 2547992	531	RBAM_RS10985	100% / 99%
7	**Bacillibactin**	**NRPS**	*ybdZ*	BVQ_16605	3292505 - 3292290	216	RBAM_RS14485	100% / 98%
			dhbF	BVQ_16610	3299651 - 3292524	7128	RBAM_RS14490	100% / 99%
			dhbB	BVQ_16615	3300592 - 3299666	927	RBAM_RS14495	100% / 98%
			dhbE	BVQ_16620	3302235 - 3300610	1626	RBAM_RS14500	100% / 99%
			dhbC	BVQ_16625	3303450 - 3302254	1197	RBAM_RS14505	100% / 99%
			dhbA	BVQ_16630	3304259 - 3303474	786	RBAM_RS14510	100% / 99%
			yuiI	BVQ_16635	3305264 - 3304395	870	RBAM_RS14515	100% / 99%
8	**Bacilysin**	**NRPS**	*ywfg*	BVQ_19710	3914189 - 3912990	1200	RBAM_RS17415	100% / 99%
			bacE	BVQ_19715	3914202 - 3915383	1182	RBAM_RS17420	100% / 99%
			bacD	BVQ_19720	3915380 - 3916798	1419	RBAM_RS17425	100% / 99%
			bacC	BVQ_19725	3917577 - 3916816	762	RBAM_RS17430	100% / 99%
			bacB	BVQ_19730	3918284 - 3917574	711	RBAM_RS17435	100% / 100%
			bacA	BVQ_19735	3918888 - 3918274	615	RBAM_RS17440	100% / 99%
			ywfA	BVQ_19740	3920287 - 3919049	1239	RBAM_RS17445	100% / 99%
Cluster number	**Metabolites**	**Synthetase**	**genes**	**Accession**	**Position**	**Bp**	***B. velezensis* CC09**	
9	**Unknown**	**TransATPKS-NRPS**	*tnrpsA*	BVQ_01170	210823 - 211518	696	A1D33_RS08065	100% / 99%
			tnrpsB	BVQ_01175	211893 - 219515	7623	A1D33_RS08060	100% / 99%
			tnrpsC	BVQ_01180	219475 - 230232	10758	A1D33_RS08055	100% / 99%
			tnrpsD	BVQ_01185	230315 - 231568	1254	A1D33_RS08050	100% / 99%
			tnrpsE	BVQ_01190	231492 - 236384	4893	A1D33_RS08045	100% / 99%
			tnrpsF	BVQ_01195	236569 - 241359	4791	A1D33_RS08040	100% / 99%
			tnrpsG	BVQ_01200	241381 - 242904	1524	A1D33_RS08035	100% / 99%
			tnrpsH	BVQ_01205*	243002 - 249628	6627	A1D33_RS08030	100% / 99%
Cluster number	**Metabolites**	**Synthetase**	**genes**	**Accession**	**Position**	**bp**	***Bacillus subtilis* strain A13**	
10	**Subtilin-like/Ericin**	**Lantipeptide**	unknown	BVQ_17650	3486343 - 3486663	321	AAL15576	100% / 100%
			eriK	BVQ_17655	3488531 - 3487212	1374	AAL15574*	98% / 100%
			eriR	BVQ_17660	3489238 - 3488576	663	AAL15573	100% / 100%
			eriG	BVQ_17665*	3490026 - 3489256	771	AAL15575	79% / 100%
			eriE	BVQ_17670	3490783 - 3490028	756	AAL15572	100% / 100%
			eriF	BVQ_17675	3491496 - 3490780	717	AAL15571*	96% / 100%
			eriI	BVQ_17680	3492004 - 3491498	507	AAL15570	100% / 100%
			eriSb	BVQ_17685	3492726 - 3492556	171	AAL15569	100% / 100%
			unknown	BVQ_17690	3492828 - 3493160	333	AAL15568*	93% / 100%
			eriSa	BVQ_17695	3493315 - 3493154	162	AAL15567*	94% / 100%
			eriC	BVQ_17700	3494713 - 3493388	1326	AAL15566	100% / 100%
			eriT	BVQ_17705	3496530 - 3494686	1845	AAL15565	100% / 100%
			eriB	BVQ_17710	3499613 - 3496521	3093	AAL15564	100% / 100%
			proZ/opuBD	BVQ_17715*	3500498 - 3499827	672	AAL15578	33% / 99%
Cluster number	**Metabolites**	**Synthetase**	**genes**	**Accession**	**Position**	**bp**	***B. velezensis CC09***	
11	**Unknown**	**NRPS**	*nrpsG*	BVQ_18400	3640781 - 3640056	726	A1D33_RS12065*	93% / 99%
			nrpsF	BVQ_18405	3641902 - 3640832	1071	A1D33_RS12060	100% / 99%
			nrpsE	BVQ_18410	3643049 - 3641988	1062	A1D33_RS12055	100% / 99%
			nrpsD	BVQ_18415	3644824 - 3643082	1743	A1D33_RS12050	100% / 99%
			nrpsC	BVQ_18420	3656339 - 3645021	11319	A1D33_RS12045	100% / 99%
			nrpsB	BVQ_18425*	3673450 - 3656351	17100	A1D33_RS12040	63% / 98%
			nrpsA	BVQ_18430*	3674486 - 3673957	530	A1D33_RS12035	86% / 99%
Cluster number	**Metabolites**	**Synthetase**	**genes**	**Accession**	**Position**	**bp**	**B. velezensis FZB42**	
12	-	**OtherKS**	-	BVQ_05095 BVQ_05305	-986643 - 1027887	41245	RBAM_RS04810 RBAM_RS05005	-100% / 99%
13	-	**T3PKS**	-	BVQ_10520 BVQ_10820	-2166999 - 2208099	41101	RBAM_RS09935 RBAM_RS08595	-65% / 99%
14	-	**Terpene**	-	BVQ_05725 BVQ_05840	-1110586 - 1131326	20741	RBAM_RS05415 RBAM_RS05525	-89% / 99%
15	-	**Terpene**	-	BVQ_09985 BVQ_10100	-2080722 - 2102605	21884	RBAM_RS09450	92% / 99%

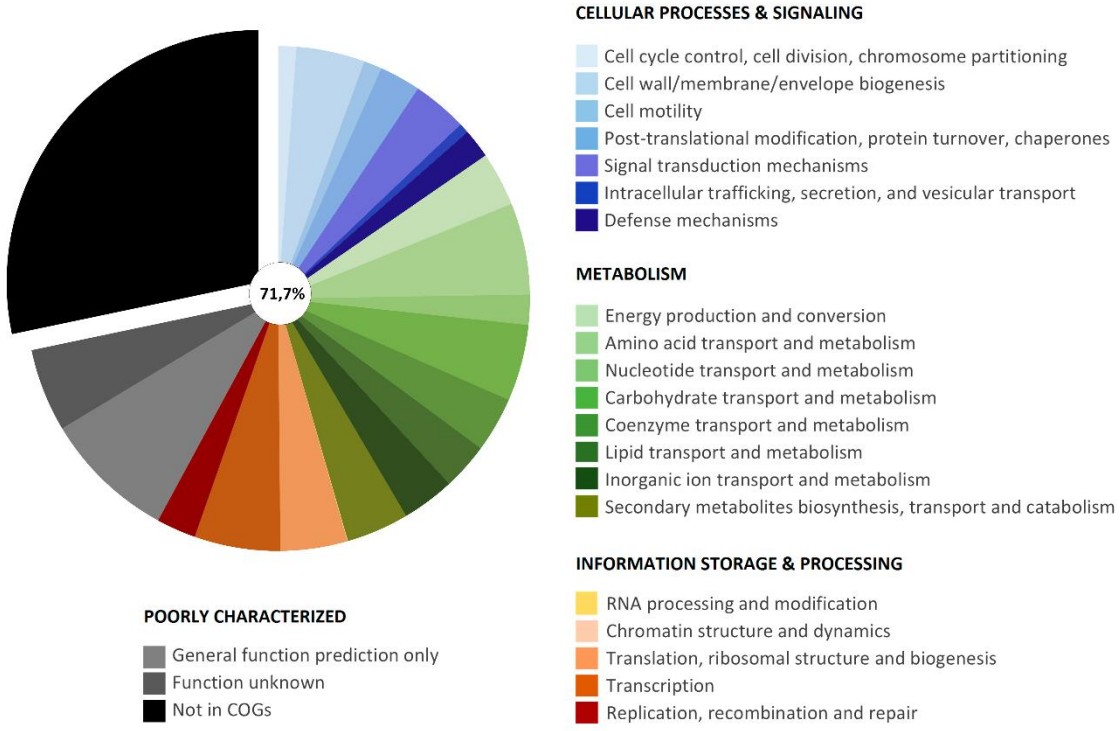

Fig. S1: Classification of genes into orthologous groups (COG) in *B. velezensis* QST713. Distribution of *B. velezensis* QST713 coding sequences (71.7%) symbolized in percentages of genes classified into COG. Genes not found in the cog annotation are classified in the category "not in COG" in black. The functional classification of protein-coding genes was performed using RPS-BLAST with COG database and classified into categories *via* the WebMGA server (Wu *et al*. 2011).

Chapitre 2: Quel impact l'agent de biocontrôle *Bacillus velezensis* QST713 a sur les communautés microbiennes du compost de culture d'*Agaricus bisporus* ?

Préambule

L'apport d'un microorganisme dans un système ne devrait pas se faire de manière anodine au risque de perturber les communautés microbiennes autochtones du système et de ce fait son fonctionnement complet. La souche QST713 utilisée depuis une dizaine d'années pour protéger les cultures du champignon de couche et nouvellement réassignée à l'espèce *Bacillus velezensis* (Partie Résultats, Chapitre 1), est mondialement utilisée pour protéger divers types de cultures, cependant peu d'études se sont interessées à son impact écologique sur les communautés microbiennes.

Dans ce projet, nous nous sommes interessés à l'impact de cet agent de biocontrôle sur les communautés microbiennes du compost au cours de la culture d'*A. bisporus*, et à son effect sur le compétiteur *T. aggressivum*. Nous avons réalisé cette étude en collaboration avec le Centre Technique du Champignon (CTC), basé à Distré dans le Maine-et-Loire, où les cultures nécessaires à cette expérience ont été réalisées. Les résultats de ce chapitre sont présentés sous forme d'un article paru dans *Biological Control*, 2018, 127 :39-54 : « Dynamics of compost microbiota during the cultivation of *Agaricus bisporus* in the presence of *Bacillus velezensis* QST713 as biocontrol agent against *Trichoderma aggressivum* ».

2.1 Article 4: "Dynamics of compost microbiota during the cultivation of *Agaricus bisporus* in the presence of *Bacillus velezensis* QST713 as biocontrol agent against *Trichoderma aggressivum*"

Caroline Pandin[a], Régis Védie[c], Thierry Rousseau[c], Dominique Le Coq[a,b], Stéphane Aymerich[a], Romain Briandet[a]

[a] Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[b] Micalis Institute, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[c] Centre Technique du Champignon, 49400 Distré, France.

Biological Control, 2018

(http://dx.doi.org/10.1016/j.biocontrol.2018.08.022)

Dynamics of compost microbiota during the cultivation of *Agaricus bisporus* in the presence of *Bacillus velezensis* QST713 as biocontrol agent against *Trichoderma aggressivum*.

Caroline Pandin[a], Régis Védie[c], Thierry Rousseau[c], Dominique Le Coq[a,b], Stéphane Aymerich[a], Romain Briandet[a]

[a]Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[b]Micalis Institute, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[c]Centre Technique du Champignon, Distré, France.

*Corresponding author.
E-mail addresses: romain.briandet@inra.fr (R. Briandet)

ARTICLE INFO

Agaricus bisporus
Biocontrol
Bacillus velezensis
Trichoderma aggressivum
16SrRNA sequencing

ABSTRACT

The edible button mushroom *Agaricus bisporus* is cultivated worldwide and appreciated for its organoleptic and nutritive qualities. It is nowadays cultivated at industrial scale in climatic units under controlled environmental conditions. Various organisms can trigger microbiological-induced losses, including *Trichoderma aggressivum* f. *europaeum*, the fungal agent of green-mould disease, which hinders the growth of *A. bisporus* mycelium. In France, the biocontrol agent *Bacillus velezensis* QST713 is used to control *T. aggressivum* and prevent production losses. Here, we evaluated the impact of *B. velezensis* QST713 on the natural microbiota of cultivation compost, artificially contaminated or not with the green-mould agent, under controlled conditions. Microbial enumeration and Miseq sequencing of 16S rRNA and ITS amplicons revealed the compost natural microbiota to be dominated by bacteria from the *Nocardiopsaceae* and *Sphingobacteriaceae* families, and fungi from the *Chaetomiaceae* family. Artificial contamination by *Trichoderma aggressivum* f. *europaeum* profoundly altered the fungal community, in contrast to *B. velezensis* QST713, which principally affected the abundance of the pathogen. This study provides new information on the dynamics, composition and structure of microbial communities of *A. bisporus* culture compost, the interactions that may occur in this ecosystem and will provide a better understanding of these biocontrol solutions for a better use of these tools.

1 Introduction

Agricultural losses due to pathogens, animals, and weeds represent approximately 26 to 40% of total production, worldwide (Teng, P. S. and Krupa, 1980; Teng, 1987; Oerke, 1999, 2006; Savary *et al*., 2012). Until recently, chemical pesticides were the most commonly used solutions in agriculture because of their radical effectiveness. However, their use has led to pollution and significant modifications of natural ecosystems (Di Poi *et al*., 2017; Uwizeyimana *et al.*, 2017; Lu and Lu, 2018; Otalvaro and Brigante, 2018; Perugini *et al*., 2018). Furthermore, a high level of microbial diversity is necessary to maintain healthy agro-ecosystems (Luan *et al*., 2015; Z. Wu *et al*., 2015). Indeed, microbial biodiversity in soil contributes to soil function, such as carbon and nitrogen cycles, plant productivity, and the control of pests and diseases (Barrios, 2007).

The EcoPhyto 2 plan, created by the French government, aims to reduce the use of such pesticides by 50% by 2025 (Ministère de l'Agriculture de l'Agroalimentaire et de la Forêt and Ministère de l'Ecologie du Developpement durable et de l'Energie, 2016), which will boost the emergence of biological control in agriculture. Biocontrol now represents 5% of the plant protection sector (IBMA France, 2017), and approximately 80% of the protection measures in the button mushroom field (*Agaricus bisporus*) in France (Personal communication: French Mushrooms Technical Centre, CTC). *Agaricus bisporus* is an edible Basidiomycota fungus of the *Agaricaceae* family. It is cultivated worldwide and appreciated for its gustative and nutritional qualities, as well as for its medicinal properties (Wasser and Weis, 1999; Díez and Alvarez, 2001; Manzi *et al*., 2001; Zhang *et al*., 2001). Nowadays, it is mainly cultivated in climatic units under controlled conditions for temperature, hygrometry, CO_2 content, and ventilation. Its culture takes approximately two months and is divided into two main phases (Fig. 1). The first is the vegetative phase, which allows development of the *Agaricus bisporus* mycelium in a culture compost, mainly composed of horse manure and wheat straw. This phase is divided into several stages: the spawning stage, consisting of seeding the compost with the *A. bisporus* mycelium; the incubation stage of 15 days at 25°C under conditions of 90 -100% hygrometry, high CO_2 content, and low ventilation; followed by a post-incubation step of one week, in which a layer of casing soil is deposited onto the compost to reduce acidity and permit the fruiting of *A. bisporus*. The second phase is fructification, which continues until the end of the cycle. At the beginning of this phase, the environmental conditions are modified (17°C, 85 to 95% hygrometry, low CO_2 content, and strong ventilation) to allow fruiting of the button mushroom (formation of sporophores) (Stamets and Chilton, 1983; Stamets and Paul, 2000; Sánchez, 2004; Eastwood *et al*., 2013; McGee, 2018). During these phases, *Agaricus* can be attacked by various pathogens which can intervene at various stages during culture, including *Trichoderma aggressivum* f. *europaeum*, responsible for green-mould disease, *Verticillium fungicola*, responsible for dry-bubble disease, and *Pseudomonas tolasii*, responsible for brown-blotch disease (Largeteau and Savoie, 2008, 2010). *Trichoderma aggressivum* f. *europaeum* Ta2 is an Ascomycota of the *Hypocreaceae* family. This filamentous fungus can decimate the production of *Agaricus*, mainly during the vegetative phase, by impeding the growth of *A. bisporus* (Mamoun *et al*., 2000; Largeteau and Savoie, 2010). This can be observed early during the fructification phase, at 28 days, by the absence of *A. bisporus* mycelium and sporophores in infected areas and the presence of a green colour, due to the sporulation of *T. aggressivum* (Krupke *et al*., 2003; Mamoun *et al*., 2000; Largeteau and Savoie, 2010). A biocontrol solution was introduced in France in the mushroom field 10 years ago and consists of the use of the commercial product SerenadeMax® (Bayer CropScience), based on *Bacillus velezensis* QST713 (formerly known as *Bacillus subtilis* QST713) (Pandin *et al*., 2018).

Bacilli are ubiquitous bacteria found in soil and a source of efficient biocontrol agents (BCAs) (Chowdhury *et al*., 2013; Lahlali *et al*., 2013; Erlacher *et al*., 2014; Liu *et al*., 2014; Wu *et al*., 2016; Al-Ali *et al*., 2017; Pandin *et al*., 2017). Indeed, bacilli can rapidly colonize crop substrates, promote plant growth, and secrete a palette of active antimicrobials (Bais, 2004; K. Wu *et al*., 2015; N. Zhang *et al*., 2015; Pandin *et al*., 2017). In addition, their biocontrol activities have been associated with their ability to form spatially organized biofilms (Al-Ali et al., 2017), with improved fitness and antagonistic activities in such environments (communication systems, niche exclusion due to spatial and nutritive competition, high-level production of antimicrobials, stress tolerance, and stimulation of plant growth and defences) (Khezri *et al*., 2011; Chen *et al*., 2013; Zeriouh *et al*., 2014; Chowdhury *et al*., 2013, 2015; Audrain *et al*., 2015; Luo *et al*., 2015; Wu et *al*., 2015; Zhang *et al*., 2015; Aleti *et al*., 2016; Pandin *et al*., 2017). The mechanisms by which *B. velezensis* QST713 antagonizes *T. aggressivum* have not yet been described, but a recent study reported the inhibition of *T. aggressivum in vitro* (Pandin *et al*., 2018). Indeed, sequence analysis of the *B. velezensis* QST713 genome showed that this strain harbours many antimicrobial clusters and an important arsenal to form complex 3D biofilms (Pandin *et al*., 2018).

The present study focused on the impact of the BCA *B. velezensis* QST713 on the button mushroom compost microbiota, artificially contaminated or not with the fungal agent of green-mould disease during the first month of the culture cycle, during which *T. aggressivum* is the most pathogenic for the mycelial growth of *A. bisporus* (Kredics *et al*., 2010). This study also provides knowledge on the persistence of the QST713 strain in compost which is reused in agricultural fields as a soil conditioner (soil fertilization, water storage, organic matter, and structure and reduction of soil pathogens) (Mehta *et al*., 2013; Alvarenga *et al*., 2017; Forte *et al*., 2017). The study of microbial community structures was performed through Illumina Miseq sequencing of the 16S rRNA gene amplicon for bacteria and internal transcribed spacer 2 (ITS2) amplicons for fungi. This analysis, under controlled conditions, improves our knowledge and understanding of the interactions and evolution of microbial communities in compost in response to the BCA *B. velezensis* QST713 applied to protect *A. bisporus* against *T. aggressivum*..

2 Materials and Methods

2.1 Experimental material and sampling design.

The evolution of microbial communities of *Agaricus bisporus* culture compost was monitored throughout the growth phase for a period of 28 days (mid-culture cycle) at the French Mushroom Technical Centre (CTC, Distré, France). Cultures were performed with (QST713 +) or without (QST713 -) treatment with *Bacillus velezensis* QST713, and with (*T. a* +) or without (*T. a* -) inoculation of *Trichoderma aggressivum* f. *europaeum* Ta2 (formerly known as *Trichoderma harzianum* Th2) (Mamoun *et al*., 2000; Largeteau and Savoie, 2010). Four conditions were studied: natural control compost (M1: *T. a* -, QST713 -), inoculated compost (M2: *T. a* +, QST713 -), treated compost (M3: *T. a* -, QST713 +), and inoculated-treated compost (M4: *T. a* +, QST713 +). Measurements were performed at four time points during the 28 days: P1 (day 0: spawning), P2 (day 7: mid-incubation), P3 (day 15: end-incubation, before casing and post-incubation), and P4 (day 28: first *A. bisporus* fructification). The compost used in this study was kindly provided by a French local producing unit (France Champignon, La Tourte, Longué-Jumelles, France). It is composed of straw and horse manure, supplemented with nitrogen (urea and ammonium sulphate), minerals (gypsum and calcium carbonate), and water. For conditions 3 and 4, the *Bacillus velezensis* QST713 BCA was added to the compost at a concentration of 0.1 $g.kg^{-1}$ of product, corresponding to 10^5 CFU (colony forming units) per gram of compost (Serenade Max, BayerCropScience, Lyon,

France). For all conditions, the compost was then seeded with *Agaricus bisporus*, at 0.8% of compost weight (Amycel Delta, white hybrid variety, Amycel, Vendôme), and divided into individual pots of 1 kg. Finally, 2 ml *Trichoderma aggressivum* suspension, at 100 spores.ml^{-1}, was added to a repository depth of 10 cm for conditions 2 and 4. Three replicates per condition were performed for P1, P2, P3, and P4 (12 pots per time point, 48 pots in total). The pots were then incubated under controlled conditions in two successive phases: (a) the vegetative phase, which started with 15 days of incubation at 25°C, followed by the addition of a 3 cm layer of casing material (60% limestone, 40% peat) supplemented with cac'ing (Amycel Delta, white hybrid variety, Amycel, Vendôme) at 112 g.m^{-2} of casing material surface and post-incubation for seven days at 25°C; and (b) the fructification phase at 17°C, in which the mushrooms emerge from the casing soil until the end of the cycle. The hygrometry during the culture was between 85 and 95%. The relative humidity of the compost at spawning and throughout the culture period was approximately 65%. Three phases of irrigation were performed on the casing soil, one after each harvest during the fructification phase. CO_2 levels were approximately 2,600 ppm from the vegetative to fructification phase and 800 to 1000 ppm from fructification to the end of the culture.

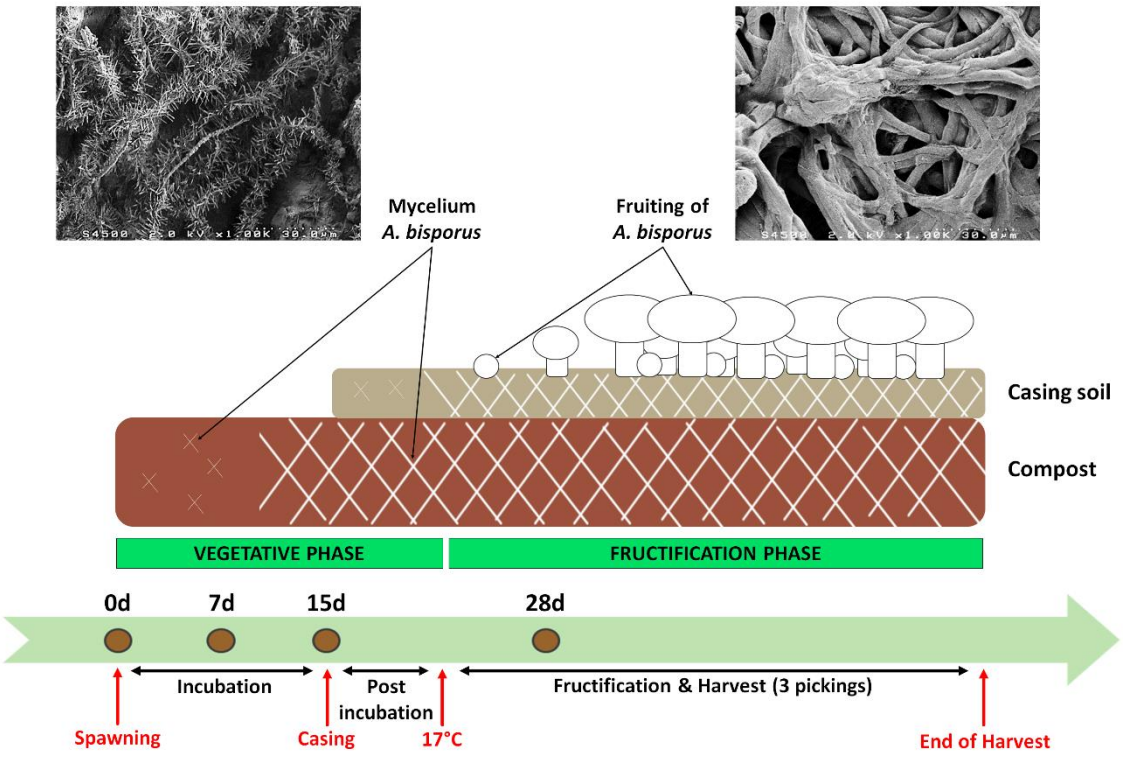

Fig. 1: Simplified culture cycle of *Agaricus bisporus* during cultivation. Scanning electron microscopy of *Agaricus bisporus* carpophore (right image), and a mycelium of *Agaricus bisporus* covered by calcium oxalate crystals in compost (left image). Samples were fixed in 0.10 M cacodylate buffer containing 2.5% (v/v) glutaraldehyde (pH 7.4) and post-fixed in a 1% osmium tetroxide solution for 1 h followed by two washings of 10 min with distilled water. Samples were then dehydrated at room temperature with increasing concentrations of ethanol (50 – 100%). Carpophore was critical point dried (Quorum Technologies K850, Elexience, France) and compost was dried with HMDS (hexamethyldisilazane). After drying, samples were mounted on grids, sputter-coated in argon plasma with platinum (Polaron SC7640, Elexience, France) and observed using a FE-SEM S4500 (Hitachi, Japan).

2.2 Microbial extraction from the compost.

For each sample (48 in total), compost was collected from each pot at the time points described above. Microorganism extraction was performed from 5 g compost in 45 mL sterile physiological saline water (0.85% NaCl) with a sterile filter bag Interscience Bag System ® (Interscience, Saint-Nom, France). The mixture was homogenized for 30 min at room temperature and then blended in a commercial laboratory blender according to the manufacturer's instructions (Seward Laboratory Lab-Blender 400, Worthing, UK). The filtered liquid was collected and readjusted to 50 ml with sterile physiological saline water.

2.3 Microbial enumeration.

Serial dilutions were made from the extracted suspensions in sterile physiological saline water (0.85% NaCl). For the enumeration of total bacterial microflora, 0.1 mL of each serial dilution was used to inoculate Trypticase Soy Agar (TSA, Biomerieux, France) supplemented with 3 $\mu g.ml^{-1}$ amphotericin B in a Petri dish. Spore-forming bacteria were enumerated from 0.1 ml of serial dilutions incubated for 10 min at 80°C and spread onto TSA medium supplemented with 3 $\mu g.ml^{-1}$ amphotericin B. *Pseudomonas* was enumerated from 0.1 mL serial dilutions plated onto cetrimide fucidin cephaloridine media (CFC; Pseudomonas agar base, supplement CFC; Sigma-Aldrich, France) supplemented with 3 $\mu g.ml^{-1}$ amphotericin B. The fungal microflora was enumerated from 0.1 ml of serial dilutions plated onto YMEA medium (2 $g.L^{-1}$ yeast extract, 20 $g.L^{-1}$malt extract, 15 $g.L^{-1}$Agar) supplemented with a mixture of citric acid (250 $\mu g.ml^{-1}$), streptomycin (100 $\mu g.ml^{-1}$), and tetracycline (50 $\mu g.ml^{-1}$). All Petri dishes were incubated at 25°C and the readings were performed after 48 h for the total bacterial microflora, spore-forming bacteria, and *Pseudomonas*, and during six days for fungi. The total bacterial microflora and *Pseudomonas* were also determined for the irrigation water and casing soil. The total bacterial microflora, spore-forming bacteria, *Pseudomonas*, and fungi on the *Agaricus bisporus* mushrooms were also analysed at the end of the culture cycle using the same protocol.

2.4 DNA extraction, Polymerase Chain Reaction and Sequencing.

A volume of 2.5 ml of previous microbial extraction from compost (corresponding to 0.25 g compost) was collected for the 48 samples and centrifuged. DNA was extracted from the pellet using a PowerLyser® PowerSoil® DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA), according to the manufacturer's instructions. Extracted DNA was quantified using a 0.9% agarose gel with a 2-Log DNA ladder (New England Biolabs) and a Qubit 3.0 Fluorometer with the Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific, Pittsburgh, PA). Bacterial V4-V5 of the 16S rRNA genes were amplified using the forward primer PCR1_515F (5′-CTTTCCCTACACGACGCTCTTCCGATC TGTGYCAGCMGCCGCGGTA-3′) and the reverse primer PCR1_928R (5′-GGAGTTCAGACGTGTGCTCTTCCGATCTC CCCGYCAATTCMTTTRAGT-3′), both containing Illumina-specific adapters. The primer sequences were provided by the GeT-PlaGe platform (Toulouse, France). The fungal internal transcribed spacer ITS2 region of the rRNA gene was amplified using the forward primer fITS7 (5'-CTTTCCCTACACGACGCTCTTCCGATC TGTGARTCATCGAATCTTTG-3') (Ihrmark *et al.*, 2012) and the reverse primer ITS4 (5'-GGAGTTCAGACGTGTGCTCTTCCGATCTT CCTCCGCTTATTGATATGC-3') (White *et al.*, 1990), both containing Illumina-specific adapters. For bacteria, PCR amplification was carried out using10 ng DNA and PCR Phusion mix (0.5 µL Phusion High-Fidelity DNA polymerase at 2 U/µL, New England Biolabs, Inc.; 10 µL 5X Phusion HF buffer containing 1.5 mM $MgCl_2$, 1 µL 10 mM dNTP mix, 2.5 µL of each primer at 10 µM, 2.5 µL bovine serum albumin (BSA) at 10 $mg.ml^{-1}$, and sterile water

added to a final volume of 50 µL). The initial enzyme activation step was performed at 98°C for 30s in a Mastercycler® thermocycler (Eppendorf AG, Hamburg, Germany), followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 40 s, and elongation for 1 min at 72°C, followed by a final elongation step at 72°C for 8 min. For fungi, PCR amplification was carried out using 50 ng DNA and PCR Phusion mix (0.5 µL Phusion High-Fidelity DNA polymerase at 2U/µL, New England Biolabs, Inc.; 10 µL 5X Phusion GC buffer containing 1.5 mM $MgCl_2$, 1 µL 10 mM dNTP mix, 1 µL of each primer at 10 µM, 2.5 µL BSA at 10 $mg.mL^{-1}$, and sterile water added to a final volume of 50 µL). The initial enzyme activation step was performed at 98°C for 1 min in a Mastercycler® thermocycler (Eppendorf AG, Hamburg, Germany), followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 53°C for 30 s, and elongation for 30 s at 72°C, followed by a final elongation step at 72°C for 7 min. The 96 PCR products were then sequenced at the GeT-PlaGe platform (Toulouse, France) using Illumina Miseq technology with the MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA).

2.5 Bioinformatics analysis and taxonomic affiliation of sequences.

Reads were analysed using the FROGS/Galaxy pipeline through the INRA MIGALE bioinformatics platform (http://migale.jouy.inra.fr) (Escudie *et al*., 2015; Afgan *et al*., 2016). For the 16S and ITS2 raw sequences, the forward and reverse primers were trimmed from the demultiplexed Illumina reads with Cutadapt (Martin, 2011; Kozich *et al*., 2013) and then read pairs were merged using Flash (Magoč and Salzberg, 2011). A total of 1,356,883 sequences was obtained for the 16S reads and 1,647,767 for the ITS2 reads. De-noising and clustering of the reads were performed with Swarm (Mahé *et al*., 2014). Chimeras were removed with the VSEARCH tool (Rognes *et al*., 2016) and 52,973 clusters were obtained from 868,197 sequences for the 16S reads and 44,988 clusters from 1,645,257 sequences for the ITS2 reads. Sequences that were present in at least three samples were kept and filtered according to abundance (> 0.01%) (Bokulich *et al*., 2013). After filtering, 342 bacterial operational taxonomic units (OTUs) were retained for affiliation processing by Blast+ and RDP classifier with the Silva128-16S reference database (Altschul *et al*., 1990; Wang *et al*., 2007), and 215 fungal OTUs for affiliation processing by Blast+ and Mycobank sequence alignment with the UNITE database (www.mycobank.org). A second filtering phase was performed, based on (i) taxonomic affiliation produced by RDP, with a minimum bootstrap percentage of 80% for bacterial and fungal OTUs, (ii) taxonomic affiliation produced by Blast, with a minimum identity of 97% and coverage of 100% for bacterial OTUs, and (iii) taxonomic affiliation produced by the Blast algorithm (www.ncbi.nlm.nih.gov/blast) and Mycobank sequence alignment (www.mycobank.org), with a minimum identity of 97% and coverage of 98% for fungal OTUs. All data were normalized through the FROGS pipeline for the same number of sequences per sample, which was the size of the smallest sample, 3,634 sequences for the 16S data and 9,325 for the ITS data. A table of OTUs for the 16S and ITS2 sequences was created, containing 342 bacterial OTUs and 193 fungal OTUs.

2.6 Button mushrooms crop yield.

A statistical study was carried out on the button mushroom crop yield data collected at the Technical Mushroom Centre (CTC, Distré, France) from February 2013 to January 2016 to determine the effect of *Bacillus velezensis* QST713 treatment on the button mushroom yield. Conditions M1 (n = 48), M2 (n = 18), M3 (n = 48) and M4 (n =18) of eight independent experiments were the same as those described above in "Experimental material and sampling design" and yield data, corresponding to the number of kg per m² of total button mushrooms, was collected.

2.7 Statistical analysis.

OTU richness, Shannon indices, and Pielou's evenness were calculated, based on the OTU table, at a depth of 3,634 sequences per sample for the 16S data and 9,325 sequences per sample for the ITS data with the "*estimate_richness*" function in the "*phyloseq*" package with Rstudio software, version 1.0.153 (Boston, USA), and plotted using XLSTAT-Premium trial software (Microsoft Corporation, USA). A hierarchical clustering plot (UPGMA) was generated using the Bray-Curtis dissimilarities distance matrix with the "*hclust*" function and a heatmap of the OTU abundance was constructed with the function "*plot_heatmap*" in the "*phyloseq*" package with Rstudio software, version 1.0.153. Metric multidimensional scaling (MDS) was performed using the Bray-Curtis dissimilarities distance matrix to explore the differences in bacterial and fungal community composition with XLSTAT-Premium trial software (Microsoft Corporation, USA). Permutational multivariate analysis (PERMANOVA) was performed using the "*adonis*" function in the "*vegan*" package in Rstudio to evaluate the significant differences between microbial community composition depending on the phase, time, and experimental condition. The Shapiro-Wilk and Levene tests were applied to determine the normality of the data distribution and homogeneity of variances. The data were not normally distributed and the variances were not homogeneous. Thus, a non-parametric Kruskal-Wallis ANOVA test and the Conover-Iman post-hoc test were performed to determine whether the observed differences were significant using XLSTAT-Premium trial software (Microsoft Corporation, USA).

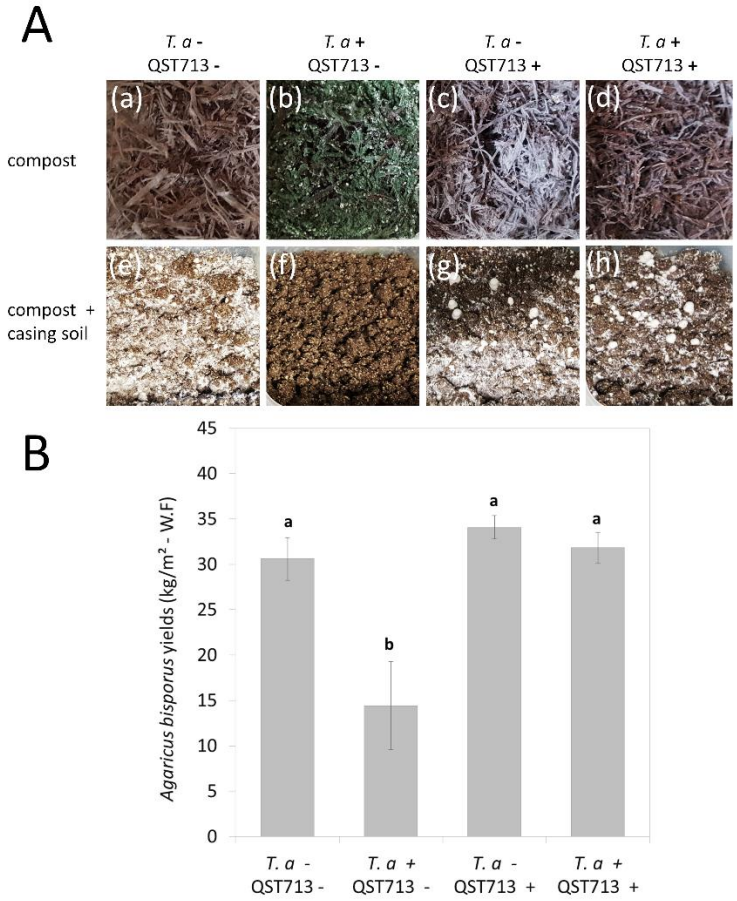

Fig. 2: Impact of inoculation of *Trichoderma aggressivum* and treatment by *B. velezensis* QST713 on *Agaricus bisporus* (A) mycelium growth (white) in the compost without casing soil at 28 days (a,b,c,d) and on the fructification of *Agaricus bisporus* in the compost with casing soil at 28 days (e,f,g,h). Treatment with *B. velezensis* QST713 (QST713 +) or not (QST713 -) and inoculation with *T. aggressivum* (*T. a* +) or not (*T. a* -). (b): invasion by *T. aggressivum* (green). (f): no *A. bisporus* mycelium development, nor fructification, (B) total yields in kg/m² of whole feet (conditions with the same letters on the graph were not significantly different).

3 Results

3.1 Effect of the biocontrol agent *Bacillus velezensis* QST713 on *Agaricus bisporus* yield.

We assessed the efficacy of the BCA *Bacillus velezensis* QST713 to protect the crop of the button mushroom, *Agaricus bisporus*. Four conditions were studied: control compost, neither inoculated with *Trichoderma aggressivum* f. *europaeum* (the pathogenic fungus of *Agaricus bisporus)* nor treated with *Bacillus velezensis* QST713 (M1: *T.a* -, QST713 -), compost inoculated with *T. aggressivum* (M2: *T.a* +, QST713 -), compost treated with *B. velezensis* QST713 (M3: *T.a* -, QST713 +), and compost inoculated with *T. aggressivum* and treated with *B. velezensis* QST713 (M4: *T.a* +, QST713 +). We observed the cultures, with or without the addition of casing soil to the compost, at 28 days (Fig. 2A). Inoculation of the culture with the pathogen *T. aggressivum* led to progressive invasion of this green mould throughout the compost, resulting in complete invasion of the compost at the fructification phase (Fig. 2A(b)) and a marked decrease in the development of *A. bisporus* mycelium and fructification relative to the other conditions (Fig. 2A(f)). In contrast, development of the mycelium of *A. bisporus* could be observed when compost inoculated with *T. aggressivum* was treated with *B. velezensis* QST713, (Fig. 2A(d)), as well as the beginning of fructification (Fig. 2A(h)). These observations were confirmed by a statistical study performed on the mushroom yield data collected from February 2013 to January 2016. The mushroom yield was significantly lower with a diminution of 52% when the compost was inoculated with *T. aggressivum* than when control compost was used ($P < 0.05$) (Fig. 2B). A normal yield was obtained when the inoculated compost was treated with *B. velezensis* QST713 ($P > 0.05$), showing the effectiveness of the BCA against *T. aggressivum*.

3.2 Evolution of culturable microbial communities of *Agaricus bisporus* compost during the crop cycle.

3.2.1 Global evolution of culturable microbial communities.

We followed the evolution of the microbial communities of *Agaricus bisporus* compost for 28 days under the same conditions as those described above (Fig. 3). At 0 days (P1), the total bacterial microflora reached an average of $1.13x10^9 \pm 1.47x10^8$ CFU (colony forming units).gr^{-1} of compost (Fig. 3A), sporulated bacteria $2.63x10^6 \pm 8.58x10^5$ CFU.gr^{-1} (Fig. 3B), *Pseudomonas* $3.68x10^2 \pm 3.27x10^1$ CFU.gr^{-1} (Fig. 3C), and fungi $3.75x10^1 \pm 1.30x10^1$ CFU.gr^{-1} (Fig. 3D), irrespective of the conditions ($P > 0.05$). The normal growth kinetics could be divided into several similar phases during the vegetative phase of *Agaricus bisporus* culture for all populations and conditions (M1, M2, M3 and M4). An increase in biomass of 1 to 5 log during the first week was followed by a decrease of 2 to 3 log until the end of the second week, except for more slowly growing fungi, for which the biomass continued to increase up to 4 log by the 15th day (Fig. 3). At the end of the vegetative phase and during the fruiting phase at 17°C, the total bacterial, sporulated, and fungal populations showed stagnation or a progressive decrease of biomass of approximately 1 to 2 log up to the 28th day. Only *Pseudomonas* showed an increase in biomass (from 2 to 3 log) up to the 28th day (Fig. 3). A search of the total bacterial microflora and *Pseudomonas* was performed on the casing soil and irrigation water to evaluate potential external contamination that could have been introduced to the system and ultimately to the *Agaricus bisporus* mushrooms harvested at the end of cultivation by leaching due to irrigation. The increase in the population of *Pseudomonas* from day 15 to day 28 was probably due to the addition of casing material, mainly composed of *Pseudomonas* ($2.79x10^4$ CFU.gr^{-1} of casing soil) and irrigation water ($1x10^1$ CFU.ml^{-1} of water) (data not shown), which renewed the population of *Pseudomonas.*

Similarly, the enumeration of microbial populations on *Agaricus bisporus* mushrooms at the end of the crop cycle revealed a microbial population similar to that of the casing soil, mainly composed of *Pseudomonas*: 98% for the control (M1) *versus* 45% for M3 and 20% for M4 (data not shown).

3.2.2 Effect of Trichoderma aggressivum f. europaeum on the culturable microbial community of compost.

The structure of the culturable microbial community studied in the presence of *T. aggressivum* was different from that of the control after 28 days. There was a significant increase of bacterial populations studied, reaching 0.5 log for *Pseudomonas* ($P = 0.007$) and sporulated bacteria ($P < 0.0001$), relative to the control compost (M1) (Fig. 3). There was also a large increase in the fungal population (exclusively *T. aggressivum*), reaching 6 logs ($P < 0.0001$), for M2. The invasion of the compost by *T. aggressivum* from the 15th day reached up to 30% of the compost population analysed at 28 days (leading to a total invasion at the end of the cycle) compared to control compost. *Pseudomonas* was also 10% more abundant than in the control compost at 28 days (data not shown).

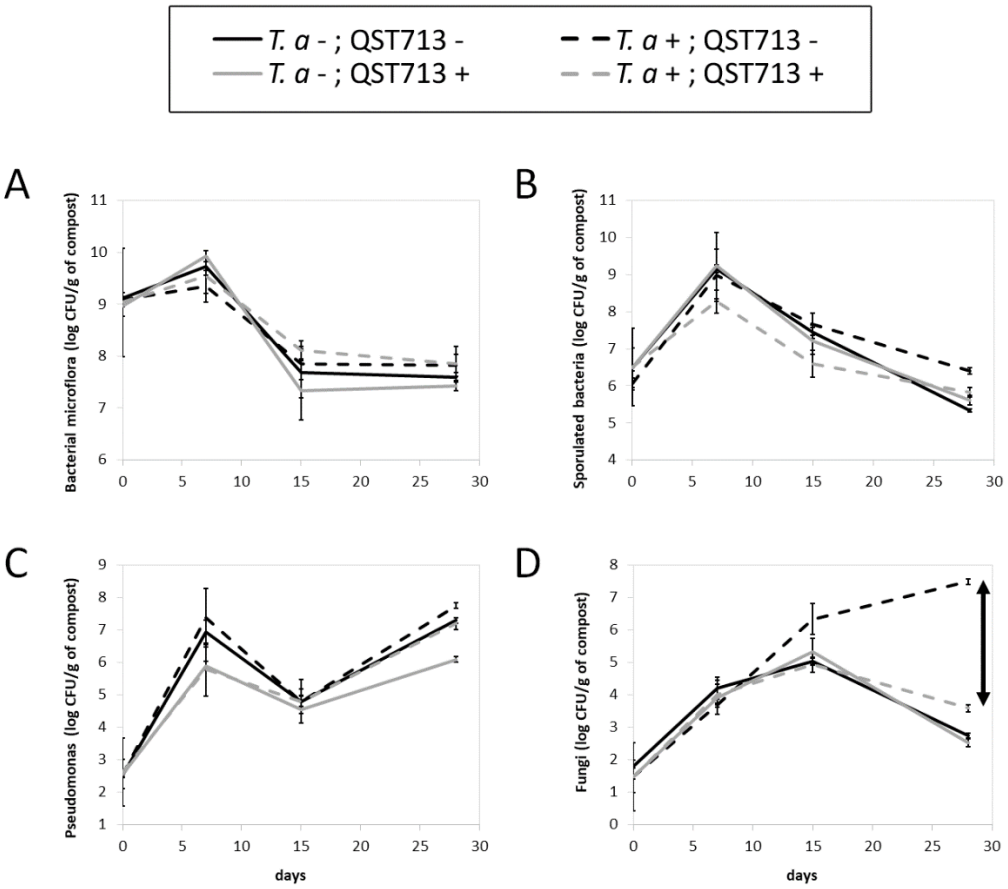

Fig. 3: Enumeration of the cultured microflora in the compost during 28 days of culture of *Agaricus bisporus*, with (QST713 +) or without (QST713 -) treatment with *Bacillus velezensis* QST713 and with (*T. a* +) or without (*T. a* -) inoculation with *Trichoderma aggressivum*. Values are the average of three repetitions with confidence intervals of 95%. (A) Cultured bacterial microflora, (B) sporulated bacteria, (C) *Pseudomonas*, and (D) fungi; cultures were counted 48 h after plating for bacteria and three to six days for fungi. Arrow represents the biocontrol effect of strain QST713. The experiment was performed in three replicates and the bars represent the 95% confidence intervals.

3.2.3 The Effect of Bacillus velezensis QST713 on the culturable microbial community of compost.

The presence of the BCA decreased the *Pseudomonas* populations by 1 log CFU.gr^{-1} (P < 0.0001) relative to the control compost by the 28th day (Fig. 3C). There was no significant effect of *B. velezensis* QST713 on the fungal populations of the treated compost (M3), which were similar to those of the control compost (M1). The treatment of compost inoculated with *T. aggressivum* by *B. velezensis* QST713 resulted in a strong decrease of the fungal population (mostly *T. aggressivum*) of approximately 2 logs CFU.gr^{-1} at 15 days (P < 0.0001) and 4 logs at 28 days (P < 0.0001) relative to the control compost (Fig. 3D), leading to a microbial community composition comparable to that of the control compost, with only slightly less spore-forming bacteria than for the other conditions.

3.3 Analysis of the α-diversity of compost microbial communities under inoculation and/or treatment.

3.3.1 α-diversity of compost bacterial communities.

The top 30 most abundant bacterial OTUs during the crop cycle are presented in Table 1. We calculated the microbial OTU richness, Shannon index, and Pielou evenness index of the compost, which are listed in Table 2. The α-diversity of the compost bacterial communities was significantly lower at the mid-incubation stage for all conditions (except M4, P > 0.05) and lower at the fructification than spawning stage (P < 0.05). At the end of the incubation stage, it was significantly lower for the control compost (P < 0.0001) and higher in the presence of the pathogen and BCA (P = 0.048). In addition, the biodiversity index was lower (P < 0.05) at the mid-incubation and fructification stage for all conditions, showing a similar temporal shift.

At the spawning stage (zero days) of the culture, the biodiversity index and Pielou's evenness indicated good bacterial biodiversity in the compost, as well as moderate uniformity of the taxa, with the four most abundant OTUs affiliated at the family level to the *Xanthomonadaceae*, *Sphingobacteriaceae*, *Nocardiopsaceae*, and *Chitinophagaceae*. At mid-incubation (seven days), the bacterial richness was significantly higher (P < 0.05) in the presence of the BCA under conditions M3 and M4 than that under conditions M1 and M2. However, this increase affected neither the biodiversity index nor the evenness within taxa (P > 0.05). At end-incubation (15 days), the bacterial richness in the presence of *T. aggressivum* (P = 0.0004), the BCA (P = 0.0001), or both (P < 0.0001) was significantly higher than that of the control compost (M1). There was also an effect on the biodiversity index, which was higher under these three conditions, whereas Pielou's evenness was only higher in the presence of the BCA, indicating that the addition of *T. aggressivum* and/or the BCA increase the biodiversity of the taxa in compost and that the BCA contributes to a better evenness of the taxa within the communities. At the fructification stage, there were no significant differences between conditions (P > 0.05), neither for OTU richness and the Shannon index nor evenness, indicating that the pathogen and the BCA have little or no impact on the richness of the bacterial communities at the late stage of culture.

3.3.2 α-diversity of compost fungal communities.

The top 30 most abundant fungal OTUs during the crop cycle are presented in Table 3. We also assessed the α-diversity of the compost fungal communities (Table 2). The evolution of the fungal richness over time was similar between the control compost (M1) and the compost inoculated with *T. aggressivum* and treated with *B. velezensis* (M4); both conditions showed significantly lower (P < 0.05) fungal richness at the mid-incubation stage, followed by an increase until the fructification stage, with higher values than in their respective controls at the spawning stage (P_{M1P1} *vs* P_{M1P4} = 0.002; P_{M4P1} *vs* P_{M4P4} = 0.049) (Table 2).

Table 1: The 30 most abundant bacterial OTUs derived from sequenced 16S amplicon DNA from throughout the cultivation process, with (QST713 +) or without (QST713 -) treatment with *Bacillus velezensis* QST713 and with (*T. a* +) or without (*T. a* -) inoculation with *Trichoderma aggressivum* for a total of four conditions: (M1) *T. a* -, QST713 -; (M2) *T. a* +, QST713 -; (M3) *T. a* -, QST713 +; (M4) *T. a* +, QST713 +

Phylum	Family	Species	OTU N°	Sampling times during the 28-day mushroom cropping process by modality and days (relative abundance %)															
				M1				M2				M3				M4			
				0	7	15	28	0	7	15	28	0	7	15	28	0	7	15	28
Bacteroidetes	*Sphingobacteriaceae*	*Sphingobacterium thermophilum*	OTU_001	12.0	41.8	22.6	0.0	4.8	27.9	5.6	0.0	12.6	42.8	2.8	0.0	10.4	33.2	3.5	0.0
Proteobacteria	*Xanthomonadaceae*	*Pseudoxanthomonas taiwanensis*	OTU_002	16.9	19.9	20.9	0.0	27.5	14.8	13.6	0.2	28.6	13.5	6.8	0.1	26.0	14.8	4.3	0.0
Actinobacteria	*Nocardiopsaceae*	*Thermobifida fusca*	OTU_003	9.3	0.4	17.8	10.1	11.8	1.2	21.6	2.8	14.1	4.0	26.4	19.3	12.7	9.1	35.4	4.5
Bacteroidetes	*Flavobacteriaceae*	*Flavobacterium aquidurense*	OTU_004	0.0	0.0	0.1	26.5	0.0	0.0	0.0	16.9	0.3	0.0	2.0	13.6	0.0	0.1	0.1	29.8
Proteobacteria	*Pseudomonadaceae*	*Pseudomonas mandelii*	OTU_005	0.0	0.1	0.1	24.2	0.0	0.1	0.0	7.3	0.4	0.0	0.0	28.5	0.0	0.0	0.1	26.6
Bacteroidetes	*Chitinophagaceae*	unknown species	OTU_006	7.6	12.7	6.4	0.0	8.9	12.0	6.1	0.0	6.9	8.6	4.4	0.0	3.7	4.5	2.1	0.0
Firmicutes	*Bacillaceae*	*Bacillus velezensis*	OTU_007	0.3	2.4	0.2	1.1	0.2	11.9	3.6	0.2	0.2	3.9	7.0	3.1	0.0	2.9	8.5	3.8
Bacteroidetes	*Chitinophagaceae*	unknown species	OTU_008	3.1	4.2	6.4	0.0	3.0	6.0	9.2	0.2	2.2	4.3	2.5	0.0	2.3	2.8	2.3	0.0
Firmicutes	*Thermoactinomycetaceae*	unknown species	OTU_009	3.5	0.0	0.6	3.9	2.8	0.0	3.5	0.1	1.8	0.9	4.6	2.5	0.3	0.6	3.4	0.2
Bacteroidetes	*Flavobacteriaceae*	*Flavobacterium sp.*	OTU_010	0.0	0.0	0.0	2.8	0.0	0.0	0.0	13.4	0.0	0.0	0.8	2.5	0.0	0.0	0.0	6.9
Proteobacteria	*Beijerinckiaceae*	*Chelatococcus sp.*	OTU_011	1.1	0.4	5.3	1.4	0.9	0.5	3.6	0.4	1.2	0.4	2.3	1.4	2.5	0.6	1.9	0.4
Firmicutes	*Planococcaceae*	unknown species	OTU_012	4.9	0.0	0.4	3.1	1.3	0.1	2.7	0.1	1.2	1.2	2.8	1.4	0.4	0.6	2.3	0.2
Bacteroidetes	*Sphingobacteriaceae*	*Pedobacter sp.*	OTU_013	0.0	0.0	0.0	1.1	0.0	0.0	0.0	19.4	0.0	0.0	0.0	0.4	0.0	0.0	0.0	1.6
Planctomycetes	*Planctomycetaceae*	*Planctomyces sp.*	OTU_014	0.6	0.2	0.8	0.3	0.2	0.1	4.4	0.3	0.9	1.5	5.1	1.4	0.2	0.3	3.9	0.3
Firmicutes	*Bacillaceae*	*Ureibacillus defluvii*	OTU_015	2.9	0.0	0.5	2.5	1.0	0.0	1.9	0.0	0.5	0.5	1.5	1.1	0.2	0.5	1.6	0.2
Bacteroidetes	*Flavobacteriaceae*	*Chryseobacterium soldanellicola*	OTU_016	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	11.3	0.0	1.8
Proteobacteria	*Moraxellaceae*	*Acinetobacter johnsonii*	OTU_017	0.0	0.0	0.0	0.0	0.0	10.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0
Proteobacteria	*Hyphomicrobiaceae*	unknown species	OTU_018	0.9	0.1	1.3	0.6	1.3	0.1	1.3	0.2	0.7	0.1	1.5	1.3	1.4	0.2	1.2	0.4
Bacteroidetes	*Flavobacteriaceae*	*Flavobacterium limicola*	OTU_019	0.0	0.0	0.0	5.0	0.0	0.0	0.0	1.8	0.1	0.0	0.2	1.8	0.0	0.0	0.0	3.6
Planctomycetes	*Planctomycetaceae*	unknown species	OTU_020	0.7	0.1	1.3	0.6	1.1	0.2	2.3	0.3	0.4	0.2	1.5	0.7	0.7	0.2	1.5	0.2
Proteobacteria	*Xanthomonadaceae*	unknown species	OTU_021	1.2	1.3	0.1	0.0	1.7	1.1	0.0	0.0	1.7	0.8	0.0	0.0	2.6	0.8	0.0	0.0
Firmicutes	*Paenibacillaceae*	unknown species	OTU_022	2.0	0.1	0.1	0.3	0.8	0.2	0.7	0.1	1.1	0.7	1.1	0.6	0.3	0.7	2.0	0.2
Firmicutes	*Bacillaceae*	*Bacillus thermolactis*	OTU_023	1.4	0.1	0.2	0.7	0.7	0.1	1.4	0.1	0.5	0.4	1.3	0.7	0.2	0.4	0.8	0.3
Firmicutes	*Planococcaceae*	*Solibacillus silvestris*	OTU_024	0.9	0.4	0.0	0.0	0.7	1.1	0.1	0.0	0.5	1.3	0.1	0.1	2.7	1.2	0.1	0.1
Actinobacteria	*Nocardiopsaceae*	*Thermobifida fusca*	OTU_025	0.7	0.0	0.4	0.4	0.6	0.1	0.9	0.2	0.7	0.3	1.3	1.1	0.2	0.3	1.7	0.2
Proteobacteria	*Alcaligenaceae*	Bordetella sp.	OTU_026	0.5	0.3	2.2	0.5	1.5	0.2	0.2	0.0	0.5	0.1	0.2	0.1	1.5	0.4	0.1	0.0
Proteobacteria	*Phyllobacteriaceae*	*Chelativorans composti*	OTU_027	1.0	0.2	0.1	0.0	1.4	0.4	0.1	0.0	1.4	0.3	0.2	0.0	2.4	0.5	0.2	0.0
Proteobacteria	*Phyllobacteriaceae*	unknown species	OTU_028	0.9	0.5	0.2	0.0	1.1	0.8	0.2	0.0	0.7	0.4	0.2	0.0	2.3	0.4	0.1	0.0
Proteobacteria	*Xanthomonadales Incertae Sedis*	unknown species	OTU_029	1.5	0.0	0.1	0.0	1.6	0.1	0.1	0.0	0.7	0.2	0.1	0.1	1.9	0.2	0.1	0.0
Acidobacteria	unknown family	unknown species	OTU_030	0.7	0.6	0.3	0.0	1.1	0.5	0.2	0.0	0.7	0.3	0.2	0.0	1.0	0.2	0.1	0.0
Accumulative abundance (%)				**74.6**	**86.0**	**88.8**	**85.2**	**75.9**	**89.7**	**83.6**	**64.2**	**80.3**	**87.0**	**77.1**	**81.8**	**76.1**	**89.3**	**77.4**	**81.4**

Table 2 : Bacterial and fungal α-diversity of *Agaricus bisporus* culture compost throughout the cultivation process with OTU richness (number of observed OTUs), Shannon index, and Pielou's evenness, with (QST713 +) or without (QST713 -) treatment with *Bacillus velezensis* QST713 and with (*T. a* +) or without (*T. a* -) inoculation with *Trichoderma aggressivum* for a total of four conditions: (M1) *T. a* -, QST713 -; (M2) *T. a* +, QST713 -; (M3) *T. a* -, QST713 +; (M4) *T. a* +, QST713 +.

Kingdom	Growth stage	Modalities	OTU richness	Shannon index	Pielou evenness
Bacteria	Spawning (P1 = 0 days)	M1	197.33 ± 8.62	3.61 ± 0.17	0.68 ± 0.03
		M2	179.67 ± 31.09	3.31 ± 0.55	0.64 ± 0.09
		M3	186.00 ± 6.56	3.12 ± 0.17	0.60 ± 0.03
		M4	149.00 ± 8.19	3.12 ± 0.36	0.62 ± 0.07
	Mid-incubation (P2 = 7 days)	M1	83.33 ± 12.90	1.95 ± 0.18	0.44 ± 0.06
		M2	103.00 ± 15.52	2.27 ± 0.09	0.49 ± 0.01
		M3	152.67 ± 7.02	2.51 ± 0.09	0.50 ± 0.01
		M4	148.33 ± 30.92	2.43 ± 0.16	0.49 ± 0.01
	End-incubation (Before post-incubation with casing soil) (P3 = 15 days)	M1	121.67 ± 19.73	2.50 ± 0.34	0.52 ± 0.05
		M2	170.67 ± 1.53	3.18 ± 0.06	0.62 ± 0.01
		M3	176.67 ± 5.86	3.33 ± 0.05	0.64 ± 0.01
		M4	176.00 ± 12.12	3.16 ± 0.22	0.61 ± 0.04
	First fructification (P4 = 28 days)	M1	139.67 ± 17.10	2.53 ± 0.38	0.51 ± 0.07
		M2	119.33 ± 18.50	2.85 ± 0.59	0.59 ± 0.11
		M3	150.00 ± 15.72	2.66 ± 0.64	0.53 ± 0.12
		M4	121.67 ± 16.17	2.41 ± 0.50	0.50 ± 0.10
Fungi	Spawning (P1 = 0 days)	M1	73.67 ± 6.81	1.52 ± 0.20	0.35 ± 0.05
		M2	73.00 ± 13.08	2.10 ± 0.28	0.49 ± 0.09
		M3	92.33 ± 10.69	1.95 ± 0.07	0.43 ± 0.01
		M4	80.33 ± 1.15	2.65 ± 0.37	0.60 ± 0.09
	Mid-incubation (P2 = 7 days)	M1	59.33 ± 14.57	0.93 ± 0.44	0.23 ± 0.10
		M2	61.00 ± 23.52	2.15 ± 0.10	0.53 ± 0.05
		M3	70.33 ± 8.74	1.73 ± 0.47	0.41 ± 0.12
		M4	77.67 ± 8.50	2.06 ± 0.35	0.47 ± 0.07
	End-incubation (Before post-incubation with casing soil) (P3 = 15 days)	M1	63.33 ± 5.13	2.32 ± 0.75	0.56 ± 0.17
		M2	71.67 ± 8.39	2.41 ± 0.22	0.57 ± 0.04
		M3	86.00 ± 7.81	2.78 ± 0.14	0.62 ± 0.02
		M4	85.67 ± 9.07	2.53 ± 0.21	0.57 ± 0.06
	First fructification (P4 = 28 days)	M1	103.00 ± 20.30	3.25 ± 0.25	0.70 ± 0.03
		M2	32.00 ± 27.78	0.38 ± 0.61	0.09 ± 0.15
		M3	97.33 ± 6.03	3.12 ± 0.05	0.68 ± 0.01
		M4	93.33 ± 0.58	3.04 ± 0.05	0.67 ± 0.01

The Shannon index revealed the same evolution for fungal biodiversity and Pielou's evenness index. However, the fungal richness evolved differently over time in the presence of *T. aggressivum* (M2) or the BCA (M3). The richness was constant until the fructification phase in the presence of *T. aggressivum*, at which time there was an abrupt decrease in richness ($P < 0.05$), as well as a decrease in the evenness of the taxa, due to the invasion of *T. aggressivum*. The richness decreased ($P < 0.05$) by the mid-incubation stage in the presence of the BCA and then increased until 15 days, followed by stabilization at the same value as that at spawning stage ($P > 0.05$). The Shannon index revealed the same evolution for the biodiversity and Pielou's evenness.

At the spawning stage, the biodiversity index and Pielou's evenness indicated poor fungal biodiversity in the compost, as well as a low uniformity of the taxa, with the four most abundant OTUs affiliated at the family level to the *Chaetomiaceae*, "Uncultured fungi", *Trichocomaceae*, and *Wallemiales incertae sedis*. At the mid-incubation stage, the fungal richness was slightly higher than that of the control compost (M1) in the presence of the pathogen and the BCA (M4) (P_{M1P2} *vs* P_{M4P2} = 0.002). The biodiversity index and Pielou's evenness were higher in the presence of *T. aggressivum* ($P_{Shannon}$

= 0.002; P_{Pielou} = 0.002) or both (*T. aggressivum* and the BCA) ($P_{Shannon}$ = 0.007; P_{Pielou} = 0.024) than in the control compost. These results suggest higher biodiversity and better uniformity of the taxa in the presence of *T. aggressivum* at the mid-incubation stage and probably a potential increase in the availability of nutrients. Pielou's evenness remained poor for all conditions, showing a predominance of some taxa over others. At the end-incubation stage, the fungal richness in M3 and M4 was significantly higher than in M1, showing a positive effect of the BCA on fungal community richness, probably due to the release of nutrients resulting from the presence of the BCA. There was better evenness at the end-incubation stage (15 days) than in any the other time points for all conditions. Finally, there were no significant differences between the conditions at the fructification stage, except in the presence of *T. aggressivum* alone (M2), for which there was a marked decrease in richness, biodiversity index, and evenness ($P < 0.05$) due to the invasion of *T. aggressivum*, as seen before. There was no significant effect on the indigenous fungal communities when the BCA alone was present (P_{M1P4} *vs* P_{M3P4} = 0.350). Treatment of the compost inoculated with *T. aggressivum* by the BCA (M4), returned the richness, Shannon index, and Pielou's evenness to the values of the control compost (P_{M1P4} *vs* $P_{M4P4} > 0.05$). They were very different from those in M2, in which *T. aggressivum* was the major species and it completely imbalanced the fungal community, showing that the BCA had a clear biocontrol effect on *T. aggressivum* and rebalanced the fungal communities. Pielou's evenness at the end-incubation (P3) and fructification stages (P4) should be interpreted cautiously for M1, M3 and M4, as *Agaricus bisporus* should logically be a major species at these stages. Bioinformatics analysis showed a low level of *Agaricus bisporus* for all conditions and all times, probably due to low extraction of its DNA because of its thick cell wall. Indeed, we observed calcium oxalate crystals covering the mycelium of *Agaricus bisporus* in the compost (Fig. 1) as previously described (Wood *et al*., 1985), which may have impeded DNA extraction.

3.4 Evolution of bacterial communities in *A. bisporus* culture compost during the cultivation process.

3.4.1 Impact of the cultivation process on bacterial communities.

We generated a heatmap with its associated hierarchical clustering plot (UPGMA), performed multidimensional scaling (MDS), based on Bray-Curtis distances, and compared the relative abundance of the 10 most abundant bacterial families to evaluate changes in the structure and composition of the bacterial communities depending on the culture phase, time, and condition (Fig. 4). The analysis revealed two major effects. The first was a culture-phase effect on the bacterial communities (P = 0.0001), corresponding to a shift in the community structures between the vegetative and fructification phase (Fig. 4AB). During the transition between these phases, many OTUs, mostly affiliated to the *Xanthomonadaceae* ($P <$ 0.0001), decreased or disappeared from the control compost (M1), along with an increase or the appearance of OTUs mostly affiliated to the *Flavobacteriaceae* ($P <$ 0.02) and *Pseudomonadaceae* (P < 0.001). The other conditions were similarly affected but with some changes in the presence of the pathogen (M2) or BCA (M3). Such appearances and disappearances may have been due to the change of incubation temperature (from 25°C to 17°C) and aeration conditions, which can favour or hinder some species. However, the casing soil added during the fructification phase could bring new species, as seen by enumeration, and reduce the acidity of the environment to promote fructification, involving a pH stress during this transition. The second major effect was a global temporal shift on microbial community structure during culture (P = 0.0001). However, it was similar for all conditions (P = 0.0597) (Fig. 4B).

Table 3: The 30 most abundant fungal OTU derived from sequenced ITS2 amplicon throughout the cultivation process, with (QST713 +) or without (QST713 -) treatment with *Bacillus velezensis* QST713 and with (*T. a* +) or without (*T. a* -) inoculation with *Trichoderma aggressivum* for a total of four conditions: (M1) *T. a* -, QST713 -; (M2) *T. a* +, QST713 -; (M3) *T. a* -, QST713 +; (M4) *T. a* +, QST713 +.

Phylum	Family	Species	OTU N°	Sampling times during the 28-day mushroom cropping process by modality and days (relative abundance %)															
				M1				M2				M3				M4			
				0	7	15	28	0	7	15	28	0	7	15	28	0	7	15	28
Ascomycota	*Chaetomiaceae*	*Mycothermus thermophilus*	OTU_001	64.2	81.8	35.6	0.6	47.2	43.4	3.7	0.1	54.3	60.0	10.2	0.3	27.9	46.8	12.5	0.1
unknown phylum	unknown family	Uncultured fungi	OTU_002	15.1	1.2	13.1	16.1	14.7	5.8	28.4	0.1	11.2	8.4	25.9	12.5	12.3	15.7	32.3	2.6
Ascomycota	*Hypocreaceae*	*Trichoderma aggressivum f. europaeum*	OTU_003	0.0	0.3	2.2	0.0	1.6	11.1	28.3	93.3	0.4	0.3	0.4	1.0	0.4	0.2	1.1	0.2
Ascomycota	*Trichocomaceae*	*Penicillium ilerdanum*	OTU_004	3.3	0.5	3.1	5.9	5.5	2.4	8.8	0.0	10.9	9.6	16.5	2.5	10.3	11.2	14.9	0.3
Basidiomycota	*Wallemiales incertae sedis*	*Wallemia sebi*	OTU_005	2.5	4.3	8.0	1.9	8.1	12.0	1.5	0.0	5.5	5.4	1.5	0.3	11.4	5.2	1.0	0.1
Ascomycota	*Saccharomycetales incertae sedis*	*Candida sp.*	OTU_006	0.0	0.1	0.5	10.0	0.0	0.2	1.1	1.2	0.3	0.0	2.3	11.8	1.8	0.0	0.3	22.8
Basidiomycota	*Agaricaceae*	*Agaricus bisporus var. bisporus*	OTU_007	0.2	4.2	11.6	4.2	0.0	1.2	2.8	0.4	0.0	0.1	3.5	1.0	0.2	0.4	1.5	1.6
unknown phylum	unknown family	Uncultured fungi	OTU_008	1.8	0.2	1.1	2.2	2.0	1.0	4.3	0.0	1.5	1.1	2.9	1.6	1.9	1.9	4.1	0.3
unknown phylum	unknown family	Uncultured fungi	OTU_009	2.3	0.2	2.6	1.2	3.2	1.6	3.1	0.0	1.0	0.7	4.3	1.9	0.9	1.4	2.1	0.2
Mucoromycota	*Mucoraceae*	*Mucor strictus*	OTU_010	0.0	0.1	0.1	2.8	0.0	0.2	0.3	1.1	0.3	0.0	0.6	7.0	3.5	0.0	0.0	6.3
Ascomycota	*Myxotrichaceae*	*Oidiodendron rhodogenum*	OTU_011	0.0	0.0	0.0	3.8	0.0	0.1	0.1	0.2	0.2	0.0	0.1	7.8	0.3	0.0	0.1	8.8
Basidiomycota	*Piskurozymaceae*	*Solicoccozyma terricola*	OTU_012	0.0	0.1	0.0	4.6	0.1	0.1	0.5	0.7	0.1	0.0	0.1	6.0	1.0	0.0	0.1	7.9
unknown phylum	unknown family	Uncultured fungi	OTU_013	1.5	0.1	1.5	1.3	0.9	0.6	2.6	0.0	1.2	1.1	2.0	1.0	1.0	1.5	2.7	0.2
unknown phylum	unknown family	Uncultured fungi	OTU_014	1.0	0.1	0.5	0.3	1.5	0.5	1.4	0.0	0.5	0.8	2.6	1.1	2.4	1.7	3.6	0.2
Ascomycota	*Hypocreaceae*	*Acrostalagmus luteoalbus*	OTU_015	0.4	0.1	0.4	0.3	0.6	0.1	0.6	0.0	0.6	0.6	1.1	0.8	1.6	1.1	7.0	0.0
Ascomycota	*Myxotrichaceae*	*Oidiodendron sp.*	OTU_016	0.0	0.0	0.0	2.3	0.0	0.1	0.4	0.3	0.1	0.0	0.2	4.6	0.2	0.0	0.0	6.8
Basidiomycota	*Wallemiales incertae sedis*	*Wallemia muriae*	OTU_017	0.8	0.9	2.0	0.4	1.7	2.4	0.2	0.0	1.6	1.1	0.2	0.0	2.9	0.7	0.1	0.0
Ascomycota	*Cladosporiaceae*	*Cladosporium herbarum*	OTU_018	0.1	1.2	2.8	4.3	1.0	1.9	0.2	0.0	0.3	0.3	0.3	0.4	1.0	0.1	0.1	0.0
Ascomycota	*Saccharomycetales incertae sedis*	*Candida subhashii*	OTU_019	0.0	0.0	0.2	2.5	0.0	0.1	0.1	0.2	0.1	0.1	0.6	3.1	0.5	0.0	0.0	5.8
Ascomycota	*Hypocreaceae*	*Acremonium sp.*	OTU_020	0.0	0.0	0.2	2.0	0.1	0.0	0.4	0.0	0.3	0.1	2.1	4.2	0.6	0.5	1.6	0.1
Ascomycota	*Debaryomycetaceae*	*Debaryomyces hansenii*	OTU_021	0.6	0.1	0.2	0.3	0.9	0.5	0.7	0.0	1.0	1.3	1.9	0.3	1.4	0.7	2.1	0.0
Ascomycota	*Saccharomycetales incertae sedis*	*Candida sp.*	OTU_022	0.0	0.0	0.1	2.1	0.0	0.1	0.3	0.1	0.1	0.0	0.3	2.8	0.6	0.0	0.0	5.3
unknown phylum	unknown family	Uncultured fungi	OTU_023	0.5	0.0	1.2	0.7	0.6	0.2	0.9	0.0	0.3	0.3	0.8	0.7	0.6	0.5	1.2	0.1
Ascomycota	*Trichomonascaceae*	*Blastobotrys malaysiensis*	OTU_024	0.0	0.0	0.0	3.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	1.8	0.1	0.0	0.1	3.0
Ascomycota	*Myxotrichaceae*	*Oidiodendron sp.*	OTU_025	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.1	0.0	0.1	2.9	0.1	0.0	0.0	3.0
Basidiomycota	*Leucosporidiaceae*	*Leucosporidium drummii*	OTU_026	0.0	0.1	0.0	1.1	0.0	0.0	0.2	0.1	0.1	0.0	0.1	2.4	1.0	0.0	0.1	2.1
Basidiomycota	*Wallemiales incertae sedis*	*Wallemia muriae*	OTU_027	0.5	0.3	0.7	0.2	1.2	1.7	0.0	0.0	0.5	0.5	0.1	0.1	0.9	0.4	0.1	0.0
Basidiomycota	*Trichosporonaceae*	*Cryptococcus sp.*	OTU_028	0.0	0.0	0.0	1.8	0.1	0.0	0.0	0.2	0.1	0.0	0.1	2.2	0.5	0.0	0.0	2.0
Ascomycota	*Pseudeurotiaceae*	*Leuconeurospora pulcherrima*	OTU_029	0.0	0.0	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.1	0.0	0.0	1.1
unknown phylum	unknown family	Uncultured fungi	OTU_030	0.5	0.0	0.6	0.3	0.8	0.5	0.6	0.0	0.3	0.1	0.8	0.3	0.1	0.3	0.6	0.0
Accumulative abundance (%)				**95.5**	**96.1**	**88.3**	**80.4**	**92.1**	**87.8**	**91.6**	**98.2**	**93.0**	**91.9**	**81.6**	**84.9**	**87.4**	**90.3**	**89.4**	**81.3**

3.4.2 Composition and evolution of bacterial communities in the control compost during culture.

The bacterial community was represented by 77 families. We selected the 10 most abundant bacterial families to monitor changes in the bacterial communities during the culture of *A. bisporus*. Four major families shaped the compost community in the control compost (M1) during the vegetative phase (P1, P2 and P3), namely the *Xanthomonadaceae*, *Sphingobacteriaceae*, *Nocardiopsaceae*, and *Chitinophagaceae*, all with a shift in abundance over time (Fig. 4C). At the spawning stage (day 0), the dominant families were the *Xanthomonadaceae* (19.10%), *Sphingobacteriaceae* (12.14%), *Nocardiopsaceae* (11.26%), and *Chitinophagaceae* (10.81%). However, this repartition was not conserved over time.

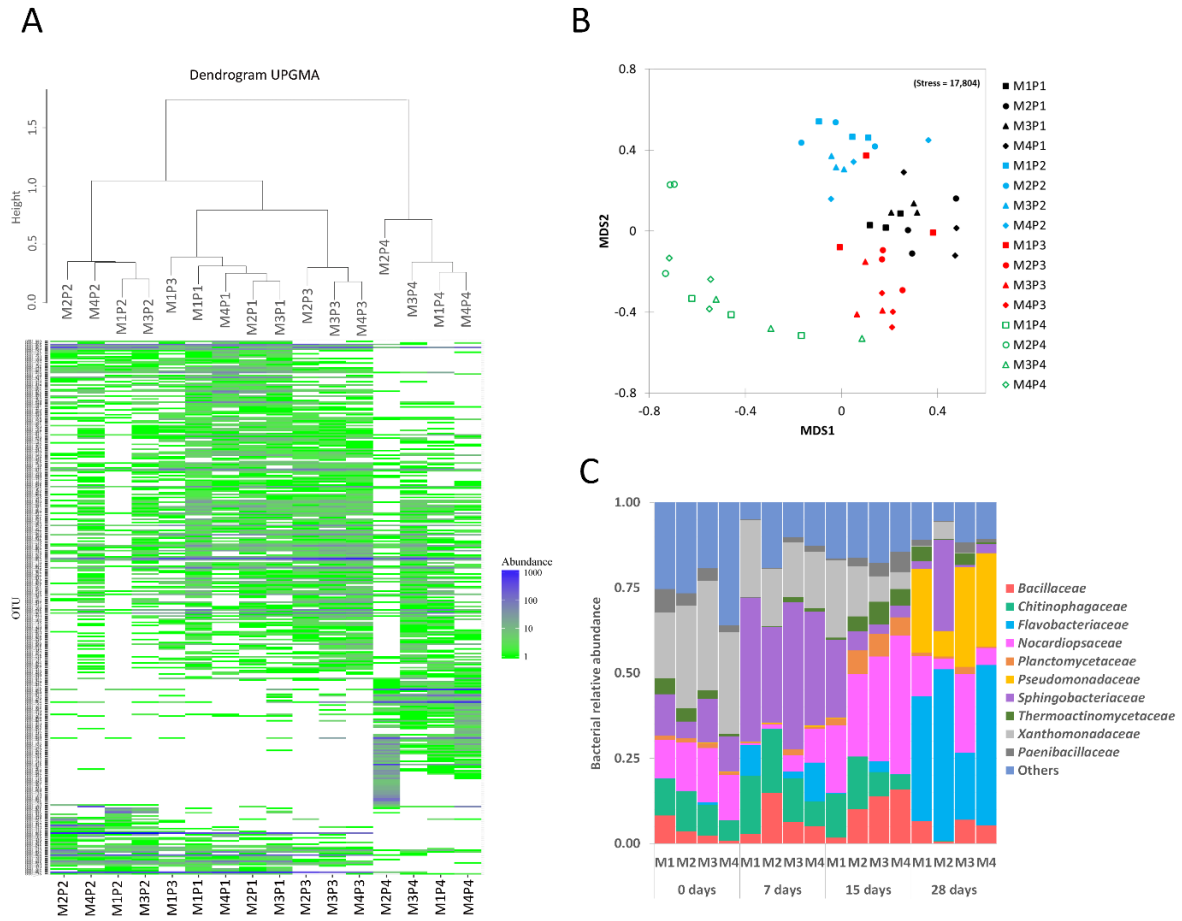

Fig. 4: Bacterial abundance *β*-diversity and community composition in compost, with or without *Bacillus velezensis* QST713 treatment and with or without inoculation with *T. aggressivum*. (A) Heatmap of the abundance of bacterial OTUs and the associated hierarchical dendrogram based on Bray-Curtis distances. (B) Multidimensional scaling based on Bray-Curtis distance representing bacterial communities, with (QST713 +) or without (QST713 -) treatment with *Bacillus velezensis* QST713 and with (*T. a* +) or without (*T. a* -) inoculation with *Trichoderma aggressivum* for a total of four conditions: (M1, square) *T. a* -, QST713 - ; (M2, circle) *T. a* +, QST713 -; (M3, triangle) *T. a* -, QST713 +; (M4, diamond) *T. a* +, QST713 +, at four time points: (P1: black) 0 days, (P2: blue) 7 days, (P3: red) 15 days, and (P4: green) 28 days. The culture phases are represented by filled squares, circles, triangles, or diamonds for the vegetative phase and empty symbols for the fructification phase. (C) Relative abundance of the 10 most abundant families of bacteria in compost under the same conditions and for the same time points as described above.

By the mid-incubation stage (day 7), the *Sphingobacteriaceae* population increased (P = 0.006) relative to that at the spawning stage and became the major family, comprising 42.28% of the community, followed by the *Xanthomonadaceae* (22.69%), *Chitinophagaceae* (17.05%), and *Nocardiopsaceae*, which dropped to 0.42% (P = 0.001). By the end-incubation stage (day 15), the four families were more uniform, with a decrease in the abundance of the *Sphingobacteriaceae* (22.82%, P < 0.0001) and an increase in that of the *Nocardiopsaceae* (19.79%, P < 0.0001), whereas those of the *Xanthomonadaceae* and *Chitinophagaceae* were unaffected (P > 0.05). By the fructification stage (day 28), the bacterial community structure was modified due to the phase effect described above, with two major families shaping the bacterial community, the *Flavobacteriaceae* (36.56%) and *Pseudomonadaceae* (24.67%).

3.4.3 Evolution of bacterial communities during the vegetative phase in the presence of T. aggressivum f. europaeum and/or B. velezensis QST713.

There were no major changes in bacterial community structure among the conditions between the spawning (day 0) and mid-incubation stages (day 7), except for an increase in the abundance of the *Bacillaceae* (P_{M2P2} = 0.021) for M2, probably in response to the development of *T. aggressivum* (Fig. 4C).

The conditions affected the bacterial community structure by the end-incubation stage (day 15) (P = 0.0053). There was an expected increase in the abundance of the *Bacillaceae* upon the addition of *B. velezensis* QST713 (P_{M3P3} = 0.0004; P_{M4P3} = 0.001), but it also occurred in the presence of *T. aggressivum* alone (P_{M2P3} = 0.008), indicating that indigenous *Bacillaceae* responded to the presence of *T. aggressivum*. The presence of *T. aggressivum* (P_{M2P3} = 0.009), *B. velezensis* QST713 (P_{M3P3} < 0.0001), or both (P_{M4P3} = 0.001) resulted in a decrease in the abundance of the *Sphingobacteriaceae*. The abundance of the *Nocardiopsaceae* had a tendency to increase in the presence of *T. aggressivum*, the BCA, or both, but the differences were not significant, probably due to variability of the data. However, Pearson correlation analysis performed on the vegetative phase (P1, P2, and P3) showed that the abundance of the *Nocardiopsaceae* and *Bacillaceae* positively correlated with the presence of the BCA (P_{M3} = 0.011; P_{M4} = 0.045), but not its absence (P_{M1} = 0.908; P_{M2} = 0.503), despite the fact that the *Bacillaceae* increased in the presence of *T. aggressivum* at the mid-incubation stage (P_{M2P2} = 0.021) (Table S1). The abundance of the *Nocardiopsaceae* also positively correlated with that of the *Hypocreaceae* (Table S1), a fungi family that was represented almost exclusively by *T. aggressivum* in this experiment. There was no significant correlation between the abundance of the *Bacillaceae* and *Sphingobacteriaceae* (P > 0.05), but the abundance of the *Nocardiopsaceae* negatively correlated with that of the *Sphingobacteriaceae* during this phase for all conditions (Table S1). In addition, the *Xanthomonadaceae* decreased significantly when the BCA was present (P_{M3P3} *vs* P_{M1P3} = 0.002; P_{M4P3} *vs* P_{M1P3}= 0.0004) and their abundance negatively correlated with that of the *Bacillaceae* in the presence of *B. velezensis* QST713 (P_{M3} = 0.001) or both (P_{M4} = 0.009) (Table S1).

The addition of a new organism, the pathogen or the BCA, appeared to induce a new competition phenomenon in this ecosystem. Furthermore, the bacterial communities showed better evenness in the control compost at the end-incubation stage. Competition for the same niche by indigenous flora might occur in compost and may be amplified by the presence of an added organism that possibly favours the *Nocardiopsaceae*, leading it to overtake the other families.

3.4.4 Evolution of bacterial communities during the fructification phase in the presence of T. aggressivum f. europaeum and/or B. velezensis QST713.

During the fructification phase (day 28), the massive appearance of the *Pseudomonadaceae*

and *Flavobacteriaceae*, after the 15th day, modified the bacterial community structure but there were no global significant differences between conditions (P = 0.0856). The evolution of the *Pseudomonas* populations studied by enumeration on specific medium agar plates did not correspond to the evolution of *Pseudomonas* found in this experiment, but rather to the succession of *Pseudoxanthomonas* during the vegetative phase and *Pseudomonas* during the fructification phase. However, there were no significant differences between conditions, in contrast to the enumeration experiment, in which there was a decrease of approximately one log under treatment with the BCA. The abundance of *Bacillaceae* was no higher when *B. velezensis* QST713 was added than that in M1, suggesting that the BCA no longer persists in compost at the fructification stage. There were no significant differences between conditions at the fructification phase, probably due to the small size (n=3) and heterogeneity of the samples. However, the abundance of *Sphingobacteriaceae* tended to be higher in the presence of *T. aggressivum* alone and that of the *Nocardiopsaceae* in the presence of the BCA alone (Fig. 4C). The evolution of the abundance of the *Sphingobacteriaceae* during the vegetative phase was opposite to that during the fructification phase in the presence of *T. aggressivum*. During the vegetative phase, *Sphingobacterium thermophilum* represented the *Sphingobacteriaceae* family, but during the fructification phase, the family was represented by *Pedobacter sp*., a species not present previously and probably introduced with the casing soil added for the fruiting of *A. bisporus*.

3.5 Evolution of fungal communities in the *A. bisporus* culture compost during the cultivation process.

3.5.1 Impact of the cultivation process and inoculation with T. aggressivum on fungal communities.

We also monitored changes in the structure and composition of fungal communities depending on culture phase, time and condition. A heatmap with UPGMA dendrogram, MDS, and the relative abundance of the 10 most abundant fungal families are shown in Fig. 5. The analysis revealed three major effects. First, there was a phase effect (P = 0.0001) between the vegetative and fructification phases (Fig. 5AB), which was less pronounced than that for the bacterial community. This effect was mainly characterized by the appearance or increase in relative abundance of OTUs, affiliated to the *Saccharomycetales incertae sedis*, *Myxotrichaceae*, and *Mucroraceae*, at the fructification stage. The effect was the same for all conditions, except M2, in which the community was invaded by the *Hypocreaceae*, exclusively *T. aggressivum* f. *europaeum*. Second, there was also a temporal shift (P = 0.0001) for the fungal communities, but later than that of the bacterial communities during the culture (Fig. 5B), probably due to many fungi growing slower than bacteria. We expected progressive growth of *A. bisporus* (*Agaricaceae*) over time until the relative abundance reached at least 45% by day 28, as described in other studies (McGee *et al*., 2017a; Zhang *et al*., 2014). However, we did not observe such an evolution, probably because the thick wall of the mycelium of *A. bisporus* prevented good DNA extraction. The third effect observed was the impact of *T. aggressivum* (M2) on communities, where it became the second most dominant family (29.97%) behind uncultured fungi (43.50%) by the end-incubation stage (day 15), and the quasi-exclusive family (93.30%) by the fructification stage (day 28) (Fig. 5C).

3.5.2 Composition and evolution of fungal communities in the control compost during culture.

The fungal community was represented by 48 identified families. We selected the 10 most abundant fungal families to monitor the evolution of fungal communities during the culture of *A. bisporus*. The dominant families shaping the fungal community of the control compost during

the vegetative phase were the *Chaetomiaceae*, *Trichocomaceae*, *Wallemiales incertae sedis*, and "Uncultured fungi" (Fig. 5C). At the spawning stage (day 0), the dominant families were the *Chaetomiaceae* (64.29%), followed by the "Uncultured fungi" (24.05%), *Trichocomaceae* (3.81 %), and *Wallemiales incertae sedis* (2.57%). This repartition was globally conserved during this phase with some changes in abundance among families. The relative abundance of the *Chaetomiaceae* was higher at the mid-incubation stage (day 7) (82.52%), followed by a decrease down to 38.19% by the end-incubation stage (day 15) (P = 0.003), unlike the "Uncultured fungi", which dropped to 2.04% (P = 0.003) by the mid-incubation stage and then increased to 22.17% by the end-incubation stage (P = 0.718). The abundance of *Wallemiales incertae sedis* increased continuously to 8.30% by the end-incubation stage (P = 0.003) (Fig. 5C). There were no significant differences in the abundance of the *Trichocomaceae*, but it tended

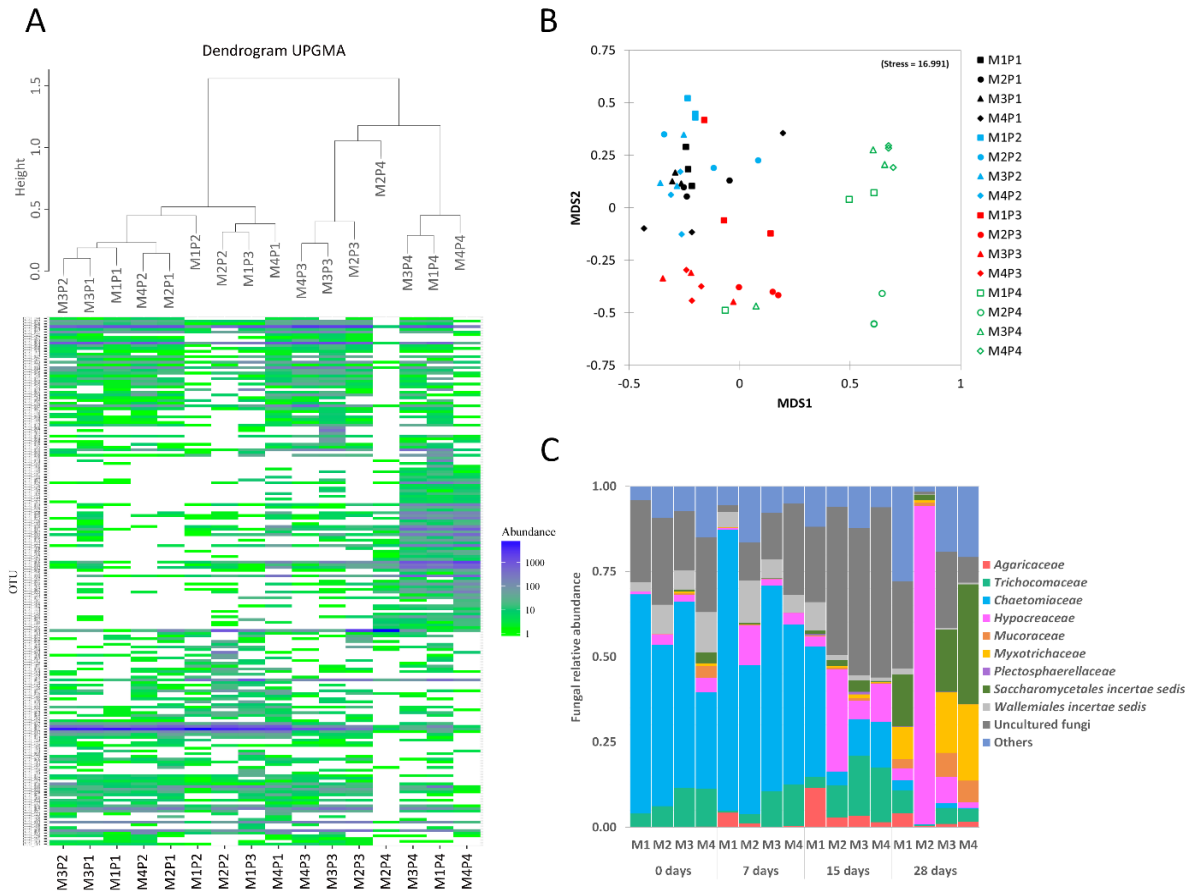

Fig. 5: Fungal abundance, *β*-diversity and community composition in compost with or without *Bacillus velezensis* QST713 treatment and with or without inoculation with *T. aggressivum*. (A) Heatmap of the abundance of fungal OTUs and the associated hierarchical dendrogram based on Bray-Curtis distances. (B) Multidimensional scaling based on Bray-Curtis distance, representing fungal communities, with (QST713 +) or without (QST713 -) treatment with *Bacillus velezensis* QST713 and with (*T. a* +) or without (*T. a* -) inoculation with *Trichoderma aggressivum* for a total of four conditions: (M1, square) *T. a* -, QST713 -; (M2, circle) *T. a* +, QST713 -; (M3, triangle) *T. a* -, QST713 +; (M4, diamond) *T. a* +, QST713 +, at four timepoints: (P1: black) 0 days, (P2: blue) 7 days, (P3: red) 15 days, and (P4: green) 28 days. The culture phases are represented by filled squares, circles, triangles, or diamonds for the vegetative phase and empty symbols for the fructification phase. (C) Relative abundance of the 10 most abundant families of fungi in compost under the same conditions and for the same time points as described above.

to be lower at the mid-incubation stage and then to return to the spawning-stage value. At the fructification stage, the structure of the community was modified due to the phase effect described above. There was better evenness in the fungal communities in the control compost by α-diversity analysis. During this phase, a rebalancing of fungal communities took place and the four major families in the control compost were the "Uncultured fungi" (25.48%), *Saccharomycetales incertae sedis* (15.20%), *Myxotrichaceae* (9.31%), and *Trichocomaceae* (6.56%).

3.5.3 Evolution of fungal communities during the vegetative phase in the presence of T. aggressivum f. europaeum and/or B. velezensis QST713.

The fungal community structure was globally similar between the spawning (day 0) and the mid-incubation stages (day 7). However, there were some significant differences at the mid-incubation stage: the *Hypocreaceae* increased when *T. aggressivum* was added (P_{M2P2} = 0.016) and the abundance of the "Uncultured fungi" was higher in the presence of *T. aggressivum* (P_{M2P2} = 0.029), *B. velezensis* QST713 (P_{M3P2} = 0.022), or both (P_{M4P2} = 0.001) than in the control compost. The abundance of the *Chaetomiaceae* was lower in the presence of *T. aggressivum* (P_{M2P2} < 0.0001), *B. velezensis* QST713 (P_{M3P2} = 0.02), or both (P_{M4P2} = 0.001) than in the control compost. The abundance of *Trichocomaceae* was higher when the BCA was added (P_{M3P2} < 0.0012; P_{M4P2} < 0.0005).

There were major differences at the end-incubation stage (day 15). The relative abundance of the "Uncultured fungi" and *Trichocomaceae* were higher in the presence of *T. aggressivum*, the BCA, or both ("Uncultured fungi": P_{M3P3} = 0.049, P_{M4P3} = 0.014; *Trichocomaceae*: P_{M2P3} = 0.01, P_{M3P3} < 0.0001, P_{M4P3} = 0.0001). In contrast, the abundance of *Chaetomiaceae* and *Wallemiales incertae sedis* was lower under these same conditions than in the control compost (*Chaetomiaceae*: P_{M2P3} = 0.0002, P_{M3P3} = 0.002, P_{M4P3} = 0.011; *Wallemiales incertae sedis*: P_{M2P3} = 0.00036, P_{M3P3} = 0.00017, P_{M4P3} < 0.0001). This evolution was confirmed by Pearson correlations during the vegetative phase, in which there were positive correlations between the abundance of "Uncultured fungi" and *Hypocreaceae* in the presence of *T. aggressivum*, (P_{M2} = 0.017) and that of the *Bacillaceae* in the presence of *T. aggressivum* (P_{M2} = 0.049), the BCA (P_{M3} = 0.021), or both (P_{M4} = 0.016) (Table S1). The abundance of *Wallemiales incertae sedis* negatively correlated with that of the *Bacillaceae* in the presence of the BCA (P_{M3} = 0.001; P_{M4} = 0.036). There was also a negative correlation between the abundance of the *Chaetomiaceae* and that of the *Hypocreaceae* in the presence of *T. aggressivum* (P_{M2} < 0.0001), the BCA (P_{M3} = 0.012), or both (P_{M4} = 0.011), and that of the *Bacillaceae* in the presence of the BCA alone (P_{M3} = 0.007) (Table S1). Under the conditions in which the BCA and the BCA/*T. aggressivum* were added, the negative correlations between the abundance of the *Chaetomiaceae* and the *Hypocreaceae* were due to the presence of other indigenous *Hypocreaceae* of the compost, such as the *Acremonium*, which increased the most in the presence of the BCA (M3P3: +3.32%; M4P3: +2.65%) by the end-incubation stage (P_{M3P3} = 0.001; P_{M4P3} = 0.001) relative to the control compost. Furthermore, there were negative correlations between the abundance of "Uncultured fungi" with that of the *Chaetomiaceae* under all conditions (Table S1), and with that of the *Wallemiales incertae sedis* in the presence of *T. aggressivum* (P_{M2} = 0.016), the BCA (P_{M3} = 0.004), or both (P_{M4} = 0.040).

These results suggest that competition might occur in the culture substrate of *A. bisporus*. The "Uncultured fungi" appeared to act similarly to the *Nocardiopsaceae* for the bacterial communities, by increasing at the late vegetative phase in the presence of an added organism. This possibility was reinforced by Pearson correlations, in which the abundance of the "Uncultured fungi" positively correlated with that of the *Nocardiopsaceae* in the presence of an

added organism during the vegetative phase (Table S1). Finally, there was an expected large increase in the abundance of *Hypocreaceae* when *T. aggressivum* was added (+27%; $P_{M2P3} = 0.004$) and to a lesser extent in the compost inoculated with *T. aggressivum* and treated with the BCA (+7%; $P_{M4P3} = 0.011$) relative to the control compost. The smaller increase in abundance under the condition in which both microorganisms were added was due to the increase in abundance of *Hypocreaceae* other than *T. aggressivum*, which were not sensitive to the biocontrol effect of *B. velezensis* QST713.

3.5.4 Evolution of fungal communities during the fructification phase in the presence of T. aggressivum f. europaeum and/or B. velezensis QST713.

During the fructification phase (28 days), there were significant differences, depending on the condition ($P = 0.005$). The major difference was the invasion of the compost by the *Hypocreaceae* when *T. aggressivum* was added alone compared to the other conditions ($P_{M2P4} < 0.048$) (Fig. 5C). The invasion of *T. aggressivum* led to a marked decrease in the abundance of almost all the populations ($P_{M2P4} < 0.027$), except the *Plectosphaerellaceae* ($P_{M2P4} = 0.513$) and the *Mucoraceae* ($P_{M2P4} = 0.170$), which both had a similar relative abundance under all conditions. For the other conditions, there were no significant differences except a decrease in the abundance of the "Uncultured fungi" in M4 ($P = 0.03$). However, the abundance of the *Saccharomycetales incertae sedis* and *Myxotrichaceae* tended to increase in the inoculated-treated compost, probably due to the release of nutrients induced by the presence of *B. velezensis* QST713 and *T. aggressivum*.

There was no significant correlation between the abundance of the *Bacillaceae* and the *Hypocreaceae* over time. The *Bacillaceae* were represented mostly by OTU_007. There was no significant correlation between the abundance of *T. aggressivum* and OTU_007 affiliated with *B. velezensis* species, including *B. velezensis* QST713 with 100% identity and coverage. This is explained by the fact that OTU_007 also included other *B. velezensis* species close to strain QST713, with 100% identity and coverage of the V4-V5 region of the 16S rRNA gene. This OTU was present under all conditions with a maximum abundance in the presence of *T. aggressivum* and the absence of QST713 (Fig. S1A), meaning that the V4-V5 region of the 16SrRNA gene did not allow differentiation between strains of *Bacillus velezensis* species. However, a comparison of the compost inoculated with *T. aggressivum* (M2) and that of the compost inoculated with *T. aggressivum* and treated with the BCA (M4), showed that the abundance of *T. aggressivum* was strongly affected when the BCA was added, with a diminution of its relative abundance of up to 98.1% by the mid-incubation stage (P_{M2P2} *vs* $P_{M4P2} = 0.035$), 96.3% by the end-incubation stage (P_{M2P3} *vs* $P_{M4P3} = 0.030$) and 99.8% by the fructification stage (P_{M2P4} *vs* $P_{M4P4} = 0.004$) relative to the compost inoculated without treatment by the BCA (Fig. S1B). These results indicate that the abundant *Bacillaceae* under the condition in which *T. aggressivum* was added alone (M2), had no effect on the abundance of *T. aggressivum.* Thus, the BCA clearly exhibited a specific inhibitory effect on *T. aggressivum* in the inoculated-treated compost (M4) that was visible as early as after seven days.

3.6 Impacts of *T. aggressivum* and/or *B. velezensis* QST713 on microbial communities.

The results described above show that *T. aggressivum*, the competitor of *Agaricus bisporus*, and *B. velezensis* QST713 had a minor impact on the bacterial communities (Fig. 6A), but *T. aggressivum* completely deconstructed the fungal communities at the fructification stage (Fig. 6B). BCA treatment induced major changes by the end-incubation stage, which diminished over time in both communities, until the microbial community structure was similar to that of the control compost at the fructification stage (Fig. 6AB). The structure of the microbial communities under conditions in which *T.*

aggressivum was treated by *B. velezensis* QST713 (M4) returned to that close to the control for both communities (Fig. 6AB).

4 Discussion

Biocontrol solutions are increasingly being used worldwide to mitigate chemical pesticides that have a harmful impact on human health and the environmental fauna and flora (Di Poi *et al*., 2017; Samtani *et al*., 2017; Uwizeyimana *et al*., 2017; Waheed *et al*., 2017; Otalvaro and Brigante, 2018; Perugini *et al*., 2018). Deciphering the biocontrol mechanisms is critical to understanding the interactions that occur in ecosystems and thus better controlling the soil pathogens in agriculture. Here, we focused on the bioprotection of *Agaricus bisporus* cultures against one of its major competitors in compost, *Trichoderma aggressivum* f. *europaeum.* The BCA *Bacillus velezensis* QST713 (Serenade®, Bayer CropSciences) has been used for almost 10 years in French mushroom production to treat green-mould disease. Its use led to a 54% improvement in mushroom yield when compost inoculated with *T. aggressivum* was treated with *B. velezensis* QST713.

Here, we demonstrated that the QST713 strain modified the structure of the compost microbiota during the early stages of culture (≤ 15 days), but that the effect did not persist. There were positive correlations between the abundance of the *Bacillaceae*, when the QST713 strain was present, and that of Actinobacteria of the *Nocardiopsaceae* family (mainly *Thermobifida fusca*) and "Uncultured fungi" not described to date. There were also negative correlations between the abundance of the *Bacillaceae*, when the QST713 strain was present, and that of Ascomycota, such as the *Chaetomiaceae*, including *Mycothermus thermophilus* and Basidiomycota, such as the *Wallemia*, mainly represented in the compost by *Wallemia sebi*. There was no direct correlation with the abundance of the *Sphingobacteriaceae* family (mostly *Sphingobacterium thermophilum*), which was the major family at the mid-incubation stage, but there was a negative correlation between the abundance of the *Nocardiopsaceae* and that of the *Sphingobacteriaceae* in the compost, which was amplified in the presence of the QST713 strain and/or *T. aggressivum*. These results show that strong competitive interactions for the same niche occur in compost.

Thermobifida fusca is an important degrader of cellulose found in the wheat straw used for the production of *Agaricus bisporus* culture compost (Oravecz *et al*., 2002; L. Zhang *et al*., 2015); cellulose degradation may release many nutrients in the compost, which may explain the increase in the richness at the end-incubation phase. *Mycothermus thermophilus* (also known as *Torula thermophila* or *Scytalidium thermophilum*) was the most abundant species until the mid-incubation stage, at which point *A. bisporus* began to spread over the compost. It is known to stimulate the growth of the mycelium of *A. bisporus* and is important for a good mushroom yield (Straatsma and Samson, 1993). Its abundance was strongly affected by *T. aggressivum* and to a lesser extent by *B. velezensis* QST713. Our study revealed that treatment with *B. velezensis* QST713 had no significant effect on *A. bisporus* yield, in the absence of the green-mould agent, in accordance with Milijašević-Marčić *et al*. (2017). *Wallemia* is a xerophilic fungi known for the spoilage of food with low water content. It is also associated with allergies and asthma, which can result, in the long-term, to farmer's lung disease (Sakamoto *et al*., 1989; Takahashi, 1997; Zalar *et al*., 2005; Lappalainen *et al*., 2012; Jančič *et al*., 2015). Its abundance in the compost was reduced by the presence of the BCA. *Sphingobacterium thermophilum* is a bacterium included in the Bacteroidetes and was recently isolated from compost and described (Yabe *et al*., 2013). However, *Sphingobacterium* is known to have a high concentration of sphingophospholipids in their cell walls (Naka *et al*., 2003) and a high capacity for the degradation and conversion of biomolecules (Lambiase, 2014); its abundance decreased in the presence of *B. velezensis*

QST713. In addition, the presence of *B. velezensis* QST713 markedly decreased the abundance of the pathogen *T. aggressivum.* This antagonistic effect was specific to this *Bacillus*, because the abundance of *T. aggressivum* was not affected by the presence of other *Bacillaceae*, in the absence of QST713. This effect was observed even before seven days of culture.

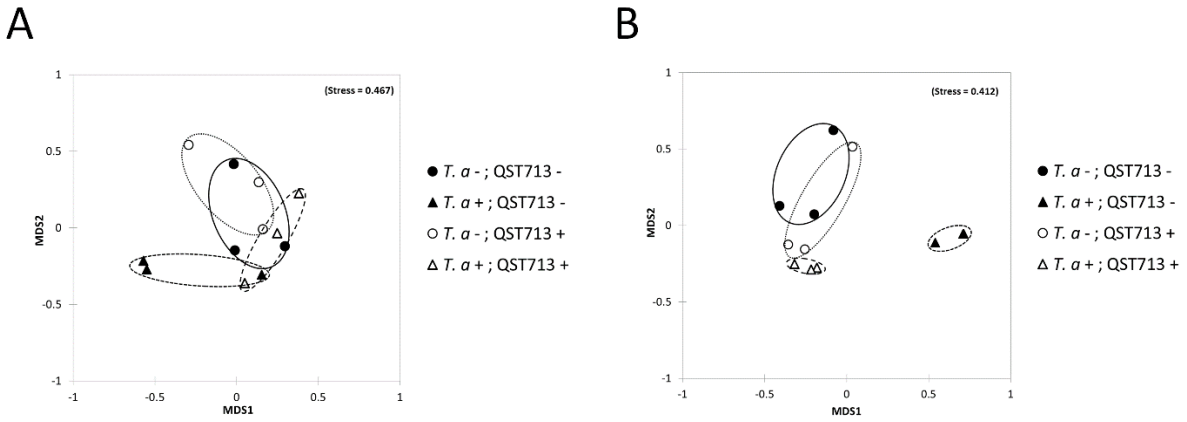

Fig. 6: Multidimensional scaling based on bray-Curtis distances of (A) bacterial and (B) fungal communities for different conditions at 28 days, with (QST713 +) or without (QST713 -) treatment with *Bacillus velezensis* QST713 and with (*T. a* +) or without (*T. a* -) inoculation with *Trichoderma aggressivum* for a total of four conditions (n = 3): (M1, filled circles) *T. a* -, QST713 -; (M2, filled triangles) *T. a* +, QST713 -; (M3, empty circles) *T. a* -, QST713 +; (M4, empty triangles) *T. a* +, QST713 +.

At the late stage of culture, the fructification phase, the QST713 strain did not appear to have any major impact on indigenous microbial communities, which were mainly composed of *Pseudomonadaceae* (mostly *Pseudomonas mandelii*), *Flavobacteriaceae* (mostly *Flavobacterium aquidurense*), uncultured fungi, *Saccharomycetales incertae sedis* (mostly *Candida sp.* and *Candida subhashii*), and *Myxotrichaceae* (mostly *Oidiodendron* genus), showing that *B. velezensis* QST713 did not persist in the compost and that it would have limited fitness in compost over time, as also observed for *B. velezensis* FZB42 in the lettuce rhizosphere (Kröber *et al.*, 2014). Modifications in composition at the fructification stage were due to the many changes between the vegetative and the fructification phase. A layer of casing soil is deposited on the compost to promote fructification by diminishing the environmental acidity (pH stress). This layer also introduces new species, which leach into the compost during irrigation and possibly compete with the species already present, causing the appearance and disappearance of some. In addition, the decrease of temperature from 25°C to 17°C, and changes in hygrometry and aeration may also favour or hinder the growth of certain microorganisms. Studies have shown that some species of *Pseudomonas* stimulate the fruiting of *A. bisporus* and that the yield is strongly affected in their absence (Rainey, 1991; Singh *et al*., 2013; Colauto *et al*., 2016). The *Flavobacteriaceae* are common Bacteroidetes of soil and water and were present mostly during the fructification phase following addition of the layer of casing soil to the compost after the 15th day of culture. They are known, like *Sphingobacteriaceae,* for their ability to produce enzymes that decompose polymers (Bernardet and Nakagawa, 2006), possibly leading to the release of new nutrients. Studies have shown that some strains of *Candida* induce

plant growth and others, such as *Candida subhashi*, can protect plants by inhibiting the development of pathogenic filamentous fungi; it is conceivable that they help protect the crop during fruiting (El-Tarabily, 2004; Hilber-Bodmer *et al*., 2017; Silambarasan and Vangnai, 2017). Recent studies have shown that some *Oidiodendron* are able to uptake nitrogen and promote plant growth by releasing ammonium (Wei *et al*., 2016). The compost is rich in nitrogen (Mcgee *et al*., 2017b) and Fermor *et al*. (1991) demonstrated that microbial cells are a concentrated source of nitrogen for *A. bisporus*, which can use ammonium and urea degraded by microorganisms. *Oidiodendron* may also have nematicidal activity (Ohtani *et al.*, 2011). Thus, the compost is rich in degraders and potential BCAs.

The BCA used in this study, *Bacillus velezensis* QST713, was previously known as *Bacillus subtilis* QST713, but a recent study on the phylogeny of the *Bacillus subtilis* species complex and recent determination of its genome sequence have led to reclassification of this strain to *Bacillus velezensis* (Fan *et al*., 2017; Pandin *et al*., 2018). *Bacilli* are ubiquitous bacteria found in soil and are powerful BCAs used in many fields (Chowdhury *et al*., 2013; Lahlali *et al*., 2013; Erlacher *et al*., 2014; Liu *et al*., 2014; Wu *et al*., 2016; Al-Ali *et al*., 2017; Pandin *et al*., 2017). Their effectiveness is based on several mechanisms which antagonize pathogens, aid in colonizing the environment, and promote plant growth (Bais, 2004; Abd El Daim *et al*., 2015; Wu *et al*., 2015; Zhang *et al*., 2015). A recent review also highlighted the advantage that the biofilm mode of life can bring to biocontrol mechanisms (Pandin *et al*., 2017). The mechanisms involved in the antagonism of *T. aggressivum* by *B. velezensis* QST713 are not yet formally described, but the QST713 strain can form highly structured biofilms and has a strong ability to inhibit the growth and sporulation of *T. aggressivum* and other pathogenic fungi (Lahlali *et al*., 2013; Pandin *et al.*, 2018). The mechanisms that antagonize *T. aggressivum* may be a combination of (i) spatial niche exclusion of *T. aggressivum* by a competitive biofilm, (ii) secretion of antifungal molecules, and (iii) secretion of signalling molecules (e.g. volatile organic compounds or quorum sensing signalling molecules) that can induce a defence response of *Agaricus bisporus* and other microorganisms in the compost. The QST713 strain is difficult to genetically manipulate and more studies are necessary to better understand its biocontrol mechanisms.

In conclusion, we observed effective inhibition of *T. aggressivum* by *B. velezensis* QST713 that possibly begins before seven days of culture, with the BCA colonizing the compost. Major changes in the composition of microbial communities occurs after 15 days of culture, when intensive competition for the same niche appears to be involved. There was no major impact on the microbial communities at the fructification phase of *A. bisporus* culture. In addition, the compost appears to be rich in degraders and potential new BCAs, which could be of value at the end of *A. bisporus* culture for soil fertilization in other fields (Ntougias *et al*., 2004; Noble and Coventry, 2005). This was suggested in a study of Mehta et *al*. (2014), which promoted the use of compost for other crops to reduce soil pathogens.

Aknowlegdments

C. Pandin is granted a doctoral fellowship by the Ile-de-France Region, DIM ASTREA (project n° ast150075). We thank the INRA MIMA2 imaging platform (www6.jouy.inra.fr/mima2) for access to microscopes and the INRA MIGALE (http://migale.jouy.inra.fr) bioinformatics platform for providing computational resources. Finally, we thank Alex Edelman and Associates for English revision of the manuscript.

References

Abd El Daim, I.A., Häggblom, P., Karlsson, M., Stenström, E., Timmusk, S., 2015. *Paenibacillus polymyxa* A26 Sfp-type PPTase inactivation limits bacterial antagonism against *Fusarium graminearum* but not of *F. culmorum*

in kernel assay. Front. Plant Sci. 06, 368. https://doi.org/10.3389/fpls.2015.00368

Afgan, E., Baker, D., van den Beek, M., Blankenberg, D., Bouvier, D., Čech, M., Chilton, J., Clements, D., Coraor, N., Eberhard, C., Grüning, B., Guerler, A., Hillman-Jackson, J., Von Kuster, G., Rasche, E., Soranzo, N., Turaga, N., Taylor, J., Nekrutenko, A., Goecks, J., 2016. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res. 44, W3–W10. https://doi.org/10.1093/nar/gkw343

Al-Ali, A., Deravel, J., Krier, F., Béchet, M., Ongena, M., Jacques, P., 2017. Biofilm formation is determinant in tomato rhizosphere colonization by *Bacillus velezensis* FZB42. Environ. Sci. Pollut. Res. 1–11. https://doi.org/10.1007/s11356-017-0469-1

Aleti, G., Lehner, S., Bacher, M., Compant, S., Nikolic, B., Plesko, M., Schuhmacher, R., Sessitsch, A., Brader, G., 2016. Surfactin variants mediate species-specific biofilm formation and root colonization in *Bacillus*. Environ. Microbiol. 18, 2634–2645. https://doi.org/10.1111/1462-2920.13405

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Alvarenga, P., Palma, P., Mourinha, C., Farto, M., Dôres, J., Patanita, M., Cunha-Queda, C., Natal-da-Luz, T., Renaud, M., Sousa, J.P., 2017. Recycling organic wastes to agricultural land as a way to improve its quality: A field study to evaluate benefits and risks. Waste Manag. 61, 582–592. https://doi.org/10.1016/j.wasman.2017.01.004

Audrain, B., Farag, M.A., Ryu, C.M., Ghigo, J.M., 2015. Role of bacterial volatile compounds in bacterial biology. FEMS Microbiol. Rev. 39, 222–233. https://doi.org/10.1093/femsre/fuu013

Bais, H.P., 2004. Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. PLANT Physiol. 134, 307–319. https://doi.org/10.1104/pp.103.028712

Barrios, E., 2007. Soil biota, ecosystem services and land productivity. Ecol. Econ. 64, 269–285. https://doi.org/10.1016/j.ecolecon.2007.03.004

Bernardet, J.-F., Nakagawa, Y., 2006. An introduction to the family *Flavobacteriaceae*, in: The Prokaryotes. Springer New York, New York, NY, pp. 455–480. https://doi.org/10.1007/0-387-30747-8_16

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59. https://doi.org/10.1038/nmeth.2276

Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R., Guo, J.H., 2013. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. Environ. Microbiol. 15, 848–864. https://doi.org/10.1111/j.1462-2920.2012.02860.x

Chowdhury, S.P., Dietel, K., Rändler, M., Schmid, M., Junge, H., Borriss, R., Hartmann, A., Grosch, R., 2013. Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. PLoS One 8, e68818. https://doi.org/10.1371/journal.pone.0068818

Chowdhury, S.P., Hartmann, A., Gao, X.W., Borriss, R., 2015. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 - A review. Front. Microbiol. 6, 780. https://doi.org/10.3389/fmicb.2015.00780

Colauto, N.B., Fermor, T.R., Eira, A.F., Linde, G.A., 2016. *Pseudomonas putida* stimulates primordia on *Agaricus bitorquis*. Curr. Microbiol. 72, 482–488. https://doi.org/10.1007/s00284-015-0982-8

Di Poi, C., Costil, K., Bouchart, V., Halm-Lemeille, M.-P., 2017. Toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. Environ. Sci. Pollut. Res. 25, 6122–6134. https://doi.org/10.1007/s11356-017-9306-9

Díez, V.A., Alvarez, A., 2001. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. Food Chem. 75, 417–422. https://doi.org/10.1016/S0308-8146(01)00229-1

Eastwood, D.C., Herman, B., Noble, R., Dobrovin-Pennington, A., Sreenivasaprasad, S., Burton, K.S., 2013. Environmental regulation of reproductive phase change in *Agaricus bisporus* by 1-octen-3-ol, temperature and CO2. Fungal Genet. Biol. 55, 54–66. https://doi.org/10.1016/j.fgb.2013.01.001

El-Tarabily, K.A., 2004. Suppression of *Rhizoctonia solani* diseases of sugar beet by antagonistic and plant growth-promoting yeasts. J. Appl. Microbiol. 96, 69–75. https://doi.org/10.1046/j.1365-2672.2003.02043.x

Erlacher, A., Cardinale, M., Grosch, R., Grube, M., Berg, G., 2014. The impact of the pathogen *Rhizoctonia solani* and its beneficial counterpart *Bacillus amyloliquefaciens* on the indigenous lettuce microbiome. Front. Microbiol. 5, 175. https://doi.org/10.3389/fmicb.2014.00175

Escudie, F., Auer, L., Bernard, M., Cauquil, L., Vidal, K., Maman, S., Mariadassou, M., Hernandez-Raquet, G., Pascal, G., 2015. FROGS: Find Rapidly OTUs with Galaxy Solution, in: The Environmental Genomic Conference, Montpellier, France. https://doi.org/10.5281/zenodo.15524

Fan, B., Blom, J., Klenk, H.P., Borriss, R., 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "operational group *B. amyloliquefaciens*" within the *B. subtilis* species complex. Front. Microbiol. 8, 22. https://doi.org/10.3389/fmicb.2017.00022

Fermor, T.R., Wood, D.A., Lincoln, S.P., Fenlon, J.S., 1991. Bacteriolysis by *Agaricus bisporus*. J. Gen. Microbiol. 137, 15–22. https://doi.org/10.1099/00221287-137-1-15

Forte, A., Fagnano, M., Fierro, A., 2017. Potential role of compost and green manure amendment to mitigate soil GHGs emissions in Mediterranean drip irrigated maize production systems. J. Environ. Manage. 192, 68–78. https://doi.org/10.1016/j.jenvman.2017.01.037

Hilber-Bodmer, M., Schmid, M., Ahrens, C.H., Freimoser, F.M., 2017. Competition assays and physiological experiments of soil and phyllosphere yeasts identify *Candida subhashii* as a novel antagonist of filamentous fungi. BMC Microbiol. 17, 4. https://doi.org/10.1186/s12866-016-0908-z

IBMA France, 2017. Le marché du biocontrôle désormais mesuré tous les ans grâce au Baromètre d'IBMA France 1–2.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Jančič, S., Nguyen, H.D.T., Frisvad, J.C., Zalar, P., Schroers, H.J., Seifert, K.A., Gunde-Cimerman, N., 2015. A taxonomic revision of the *Wallemia sebi* species complex. PLoS One 10, e0125933. https://doi.org/10.1371/journal.pone.0125933

Khezri, M., Ahmadzadeh, M., Jouzani, G.S., Behboudi, K., Ahangaran, A., Mousivand, M., Rahimian, H., 2011. Characterization of some biofilm-forming *Bacillus subtilis* strains and evaluation of their biocontrol potential against *Fusarium culmorum*. J. Plant Pathol. 93, 373–382. https://doi.org/10.4454/JPP.V93I2.1192

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120. https://doi.org/10.1128/AEM.01043-13

Kredics, L., García Jimenez, L., Naeimi, S., Czifra, D., Urbán, P., Manczinger, L., Vágvölgyi, C., Hatvani, L., 2010. A challenge to mushroom growers: the green mould disease of cultivated champignons. Appl. Microbiol. Microb. Biotechnol. 295–305.

Kröber, M., Wibberg, D., Grosch, R., Eikmeyer, F., Verwaaijen, B., Chowdhury P., S.P., Hartmann, A., Pöhler, A., Schlöter, A., 2014. Effect of the strain *Bacillus amyloliquefaciens* FZB42 on the microbial community in the rhizosphere of lettuce under field conditions analyzed by whole metagenome sequencing. Front. Microbiol. 5, 252. https://doi.org/10.3389/fmicb.2014.00252

Krupke, O.A., Castle, A.J., Rinker, D.L., 2003. The North American mushroom competitor, *Trichoderma aggressivum* f. *aggressivum*, produces antifungal compounds in mushroom compost that inhibit mycelial growth of the commercial mushroom *Agaricus bisporus*. Mycol. Res. 107, 1467–1475. https://doi.org/10.1017/S0953756203008621

Lahlali, R., Peng, G., Gossen, B.D., McGregor, L., Yu, F.Q., Hynes, R.K., Hwang, S.F., McDonald, M.R., Boyetchko, S.M., 2013. Evidence that the biofungicide serenade (*Bacillus subtilis*) suppresses clubroot on canola via antibiosis and induced host resistance. Phytopathology 103, 245–254. https://doi.org/10.1094/PHYTO-06-12-0123-R

Lambiase, A., 2014. The family *Sphingobacteriaceae*, in: The Prokaryotes: Other Major Lineages of Bacteria and the Archaea. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 907–914. https://doi.org/10.1007/978-3-642-38954-2_136

Lappalainen, M.H.J., Hyvrinen, A., Hirvonen, M.R., Rintala, H., Roivainen, J., Renz, H., Pfefferle, P.I., Nevalainen, A., Roponen, M., Pekkanen, J., 2012. High indoor microbial levels are associated with reduced Th1 cytokine secretion capacity in infancy. Int. Arch. Allergy Immunol. 159, 194–203. https://doi.org/10.1159/000335596

Largeteau, M.L., Savoie, J.M., 2010. Microbially induced diseases of *Agaricus bisporus*: Biochemical mechanisms and impact on commercial mushroom production. Appl. Microbiol. Biotechnol. 86, 63–73. https://doi.org/10.1007/s00253-010-2445-2

Largeteau, M.L., Savoie, J.M., 2008. Effect of the fungal pathogen *Verticillium fungicola* on fruiting initiation of its host, *Agaricus bisporus*. Mycol. Res. 112, 825–828. https://doi.org/10.1016/j.mycres.2008.01.018

Liu, Y., Zhang, N., Qiu, M., Feng, H., Vivanco, J.M., Shen, Q., Zhang, R., 2014. Enhanced rhizosphere colonization of beneficial *Bacillus amyloliquefaciens* SQR9 by pathogen infection. FEMS Microbiol. Lett. 353, 49–56. https://doi.org/10.1111/1574-6968.12406

Lu, X.M., Lu, P.Z., 2018. Response of microbial communities to pesticide residues in soil restored with *Azolla imbricata*. Appl. Microbiol. Biotechnol. 102, 475–484. https://doi.org/10.1007/s00253-017-8596-7

Luan, F.G., Zhang, L.L., Lou, Y.Y., Wang, L., Liu, Y.N., Zhang, H.Y., 2015. Analysis of microbial diversity and niche in rhizosphere soil of healthy and diseased cotton at the flowering stage in southern Xinjiang. Genet. Mol. Res. 14, 1602–1611. https://doi.org/10.4238/2015.March.6.7

Luo, C., Liu, X., Zhou, H., Wang, X., Chen, Z., 2015. Nonribosomal peptide synthase gene clusters for lipopeptide biosynthesis in Bacillus subtilis 916 and their phenotypic functions. Appl. Environ. Microbiol. 81, 422–431. https://doi.org/10.1128/AEM.02921-14

Magoč, T., Salzberg, S.L., 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies.

Bioinformatics 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Mahé, F., Rognes, T., Quince, C., de Vargas, C., Dunthorn, M., 2014. Swarm: robust and fast clustering method for amplicon-based studies. PeerJ 2, e593. https://doi.org/10.7717/peerj.593

Mamoun, M.L., Savoie, J.M., Olivier, J.M., 2000. Interactions between the pathogen *Trichoderma harzianum* Th2 and *Agaricus bisporus* in mushroom compost. Mycologia 92, 233–240. https://doi.org/10.2307/3761556

Manzi, P., Aguzzi, A., Pizzoferrato, L., 2001. Nutritional value of mushrooms widely consumed in Italy. Food Chem. 73, 321–325. https://doi.org/10.1016/S0308-8146(00)00304-6

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10. https://doi.org/10.14806/ej.17.1.200

McGee, C.F., 2018. Microbial ecology of the Agaricus bisporus mushroom cropping process. Appl. Microbiol. Biotechnol. 102, 1075–1083. https://doi.org/10.1007/s00253-017-8683-9

McGee, C.F., Byrne, H., Irvine, A., Wilson, J., 2017a. Diversity and dynamics of the DNA-and cDNA-derived compost fungal communities throughout the commercial cultivation process for Agaricus bisporus. Mycologia 109, 475–484. https://doi.org/10.1080/00275514.2017.1349498

Mcgee, C.F., Byrne, H., Irvine, A., Wilson, J., 2017b. Diversity and dynamics of the DNA and cDNA-derived bacterial compost communities throughout the *Agaricus bisporus* mushroom cropping process. Ann. Microbiol. 67, 751–761. https://doi.org/10.1007/s13213-017-1303-1

Mehta, C.M., Gupta, V., Singh, S., Srivastava, R., Sen, E., Romantschuk, M., Sharma, A.K., 2013. Role of microbiologically rich compost in reducing biotic and abiotic stresses. Microorg. Environ. Manag. Microbes Environ. 113–134. https://doi.org/10.1007/978-94-007-2229-3_5

Mehta, C.M., Palni, U., Franke-Whittle, I.H., Sharma, A.K., 2014. Compost: Its role, mechanism and impact on reducing soil-borne plant diseases. Waste Manag. 34, 607–622. https://doi.org/10.1016/j.wasman.2013.11.012

Milijašević-Marčić, S., Stepanović, M., Todorović, B., Duduk, B., Stepanović, J., Rekanović, E., Potočnik, I., 2017. Biological control of green mould on *Agaricus bisporus* by a native *Bacillus subtilis* strain from mushroom compost. Eur. J. Plant Pathol. 148, 509–519. https://doi.org/10.1007/s10658-016-1107-3

Ministère de l'Agriculture de l'Agroalimentaire et de la Forêt, Ministère de l'Ecologie du Developpement durable et de l'Energie, 2016. Plan Ecophyto II - Agro-écologie, produisons autrement. Agro-écologie Produisons Autrem. 1–66.

Naka, T., Fujiwara, N., Yano, I., Maeda, S., Doe, M., Minamino, M., Ikeda, N., Kato, Y., Watabe, K., Kumazawa, Y., Tomiyasu, I., Kobayashi, K., 2003. Structural analysis of sphingophospholipids derived from *Sphingobacterium spiritivorum*, the type species of genus *Sphingobacterium*. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1635, 83–92. https://doi.org/10.1016/j.bbalip.2003.10.010

Noble, R., Coventry, E., 2005. Suppression of soil-borne plant diseases with composts: A review. Biocontrol Sci. Technol. 15, 3–20. https://doi.org/10.1080/09583150400015904

Ntougias, S., Zervakis, G.I., Kavroulakis, N., Ehaliotis, C., Papadopoulou, K.K., 2004. Bacterial diversity in spent mushroom compost assessed by amplified rDNA restriction analysis and sequencing of cultivated isolates. Syst. Appl. Microbiol. 27, 746–754. https://doi.org/10.1078/0723202042369857

Oerke, E.-C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31. https://doi.org/10.1017/S0021859605005708

Oerke, E.-C., 1999. Estimated crop losses due to pathogens, animal pests and weeds, in: Crop Production and Crop Protection. Elsevier, pp. 72–741. https://doi.org/10.1016/B978-0-444-82095-2.50009-9

Ohtani, K., Kawano, T., Kimura, Y., Fujioka, S., Shimada, A., 2011. Nematicidal activities of 4-hydroxyphenylacetic acid and oidiolactone D produced by the fungus *Oidiodendron* sp. Zeitschrift fur Naturforsch. - Sect. C J. Biosci. 66, 31–34. https://doi.org/10.1515/znc-2011-1-205

Oravecz, O., Hornok, L., Nagy, I., Tóth, E., Kukolya, J., Láday, M., Márialigeti, K., 2002. *Thermobifida cellulolytica* sp. *nov.*, a novel lignocellulose-decomposing actinomycete. Int. J. Syst. Evol. Microbiol. 52, 1193–1199. https://doi.org/10.1099/00207713-52-4-1193

Otalvaro, J.O., Brigante, M., 2018. Interaction of pesticides with natural and synthetic solids. Evaluation in dynamic and equilibrium conditions. Environ. Sci. Pollut. Res. 25, 6707–6719. https://doi.org/10.1007/s11356-017-1020-0

Pandin, C., Le Coq, D., Canette, A., Aymerich, S., Briandet, R., 2017. Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? Microb. Biotechnol. 10, 719–734. https://doi.org/10.1111/1751-7915.12693

Pandin, C., Le Coq, D., Deschamps, J., Védie, R., Rousseau, T., Aymerich, S., Briandet, R., 2018. Complete genome sequence of *Bacillus velezensis* QST713: a biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease. J. Biotechnol. 278, 10–19. https://doi.org/10.1016/j.jbiotec.2018.04.014

Perugini, M., Tulini, S.M.R., Zezza, D., Fenucci, S., Conte, A., Amorena, M., 2018. Occurrence of agrochemical residues in beeswax samples collected in Italy during 2013–2015. Sci. Total Environ. 625, 470–476. https://doi.org/10.1016/j.scitotenv.2017.12.321

Rainey, P.B., 1991. Effect of *Pseudomonas putida* on hyphal growth of *Agaricus bisporus*. Mycol. Res. 95, 699–704. https://doi.org/10.1016/S0953-7562(09)80817-4

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584

Sakamoto, T., Urisu, A., Yamada, M., Matsuda, Y., Tanaka, K., Torii, S., 1989. Studies on the osmophilic fungus *Wallemia sebi* as an allergen evaluated by skin prick test and radioallergosorbent test. Int. Arch. Allergy Immunol. 90, 368–372. https://doi.org/10.1159/000235055

Samtani, R., Sharma, N., Garg, D., 2017. Effects of endocrine-disrupting chemicals and epigenetic modifications in ovarian cancer: a review. Reprod. Sci. 25, 7–18. https://doi.org/10.1177/1933719117711261

Sánchez, C., 2004. Modern aspects of mushroom culture technology. Appl. Microbiol. Biotechnol. 64, 756–762. https://doi.org/10.1007/s00253-004-1569-7

Savary, S., Ficke, A., Aubertot, J.N., Hollier, C., 2012. Crop losses due to diseases and their implications for global food production losses and food security. Food Secur. 4, 519–537. https://doi.org/10.1007/s12571-012-0200-5

Silambarasan, S., Vangnai, A.S., 2017. Plant-growth promoting *Candida* sp. AVGB4 with capability of 4-nitroaniline biodegradation under drought stress. Ecotoxicol. Environ. Saf. 139, 472–480. https://doi.org/10.1016/j.ecoenv.2017.02.018

Singh, P.K., Lal, A., Sharma, S.K., Simon, S., 2013. Influence of *Pseudomonas putida* on the yield of *Agaricus bisporus* (Lange) Imbach. Internat. J. Plant Protec. 6, 2013.

Stamets, P., Chilton, J.S., 1983. The mushroom cultivator: a practical guide to growing mushrooms at home. S. Cal. L. Rev. 416. https://doi.org/10.1002/mus.23999

Stamets, Paul, 2000. Growing gourmet & medicinal mushrooms : a companion guide to The Mushroom cultivator 614.

Straatsma, G., Samson, R.A., 1993. Taxonomy of *Scytalidium thermophilum*, an important thermophilic fungus in mushroom compost. Mycol. Res. 97, 321–328. https://doi.org/10.1016/S0953-7562(09)81129-5

Takahashi, T., 1997. Airborne fungal colony-forming units in outdoor and indoor environments in Yokohama, Japan. Mycopathologia 139, 23–33. https://doi.org/10.1023/A:1006831111595

Teng, P. S. and Krupa, S. V., 1980. Assessment of losses which constrain production and crop improvement in agriculture and forestry, in: E. C. Stackman Commemorative Symposium. Dept. of Plant Pathology, University of Minnesota, p. 327.

Teng, P.S., 1987. Crop loss assessment and pest management, in: American Phytopathological Society. American Phytopathological Society, p. 270.

Uwizeyimana, H., Wang, M., Chen, W., Khan, K., 2017. The eco-toxic effects of pesticide and heavy metal mixtures towards earthworms in soil. Environ. Toxicol. Pharmacol. 55, 20–29. https://doi.org/10.1016/j.etap.2017.08.001

Waheed, S., Halsall, C., Sweetman, A.J., Jones, K.C., Malik, R.N., 2017. Pesticides contaminated dust exposure, risk diagnosis and exposure markers in occupational and residential settings of Lahore, Pakistan. Environ. Toxicol. Pharmacol. 56, 375–382. https://doi.org/10.1016/j.etap.2017.11.003

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wasser, S.P., Weis, A.L., 1999. Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (review). Int. J. Med. Mushrooms 1, 31–62. https://doi.org/10.1615/IntJMedMushrooms.v1.i1.30

Wei, X., Chen, J., Zhang, C., Pan, D., 2016. A new *Oidiodendron maius* strain isolated from *Rhododendron fortunei* and its effects on nitrogen uptake and plant growth. Front. Microbiol. 7, 1327. https://doi.org/10.3389/fmicb.2016.01327

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: PCR Protocols. Elsevier, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wood, D.A., Craig, G.D., Atkey, P.T., Newsam, R.J., Gull, K., Newsarn, R.J., Jll, K.G., 1985. Ultrastructural studies on the cultivation processes and growth and development of the cultivated mushroom *Agaricus bisporus*. Food Struct. 4, 143–164.

Wu, B., Wang, X., Yang, L., Yang, H., Zeng, H., Qiu, Y., Wang, C., Yu, J., Li, J., Xu, D., He, Z., Chen, S., 2016. Effects of *Bacillus amyloliquefaciens* ZM9 on bacterial wilt and rhizosphere microbial communities of tobacco. Appl. Soil Ecol. 103, 1–12. https://doi.org/10.1016/j.apsoil.2016.03.002

Wu, K., Fang, Z., Guo, R., Pan, B., Shi, W., Yuan, S., Guan, H., Gong, M., Shen, B., Shen, Q., 2015. Pectin enhances bio-control efficacy by inducing colonization and secretion of secondary metabolites by *Bacillus amyloliquefaciens* SQY 162 in the rhizosphere of tobacco. PLoS One 10, e0127418. https://doi.org/10.1371/journal.pone.0127418

Wu, Z., Hao, Z., Zeng, Y., Guo, L., Huang, L., Chen, B., 2015. Molecular characterization of microbial communities in the rhizosphere soils and roots of diseased and healthy *Panax notoginseng*. Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 108, 1059–1074. https://doi.org/10.1007/s10482-015-0560-x

Yabe, S., Aiba, Y., Sakai, Y., Hazaka, M., Kawahara, K., Yokota, A., 2013. *Sphingobacterium thermophilum* sp. *nov*., of the phylum Bacteroidetes, isolated from compost. Int. J. Syst. Evol. Microbiol. 63, 1584–1588. https://doi.org/10.1099/ijs.0.042481-0

Zalar, P., Sybren de Hoog, G., Schroers, H.J., Frank, J.M., Gunde-Cimerman, N., 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, cl. et ord. nov.). Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 87, 311–328. https://doi.org/10.1007/s10482-004-6783-x

Zeriouh, H., de Vicente, A., Pérez-García, A., Romero, D., 2014. Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. Environ. Microbiol. 16, 2196–2211. https://doi.org/10.1111/1462-2920.12271

Zhang, L., Ma, H., Zhang, H., Xun, L., Chen, G., Wang, L., 2015. *Thermomyces lanuginosus* is the dominant fungus in maize straw composts. Bioresour. Technol. 197, 266–275. https://doi.org/10.1016/j.biortech.2015.08.089

Zhang, M., Cheung, P.C.K., Zhang, L., 2001. Evaluation of mushroom dietary fiber (Nonstarch Polysaccharides) from sclerotia of *Pleurotus tuber-regium* (Fries) singer as a potential antitumor agent. J. Agric. Food Chem. 49, 5059–5062. https://doi.org/10.1021/jf010228l

Zhang, N., Yang, D., Wang, D., Miao, Y., Shao, J., Zhou, X., Xu, Z., Li, Q., Feng, H., Li, S., Shen, Q., Zhang, R., 2015. Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates. BMC Genomics 16, 685. https://doi.org/10.1186/s12864-015-1825-5

Zhang, X., Zhong, Y., Yang, S., Zhang, W., Xu, M., Ma, A., Zhuang, G., Chen, G., Liu, W., 2014. Diversity and dynamics of the microbial community on decomposing wheat straw during mushroom compost production. Bioresour. Technol. 170, 183–195. https://doi.org/10.1016/j.biortech.2014.07.093

Table S1: Negative and Positive correlations among dominant microbial families for different conditions during the vegetative phase (0, 7, and 15 days), with (QST713 +) or without (QST713 -) *Bacillus velezensis* QST713 treatment and with (*T. a* +) or without (*T. a* -) inoculation with *Trichoderma aggressivum* for a total of four conditions: (M1) *T. a* -, QST713 - ; (M2) *T. a* +, QST713 -; (M3) *T. a* -, QST713 +; and (M4) *T. a* +, QST713 +. The data described here followed a normal distribution and Pearson correlations were performed

Modalities		**M1**			**M2**			**M3**			**M4**		
Pearson Correlation		**Corr**	**R²**	***P* value**	**Corr**	**R²**	***P* value**	**Corr**	**R²**	***P* value**	**Corr**	**R²**	***P* value**
Negative													
Between bacteria	*Bacillaceae vs Xanthomonadaceae*	-0.423	0.179	0.296	-0.641	0.411	0.063	-0.902	0.813	**0.001**	-0.807	0.651	**0.009**
	Nocardiopsaceae vs Sphingobacteriaceae	-0.655	0.430	**0.055**	-0.816	0.666	**0.007**	-0.892	0.795	**0.001**	-0.702	0.493	**0.035**
	Nocardiopsaceae vs Xanthomonadaceae	0.291	0.084	0.527	-0.423	0.179	0.344	-0.426	0.182	0.292	-0.820	0.672	**0.013**
Between Fungi	*Chaetomiaceae vs Hypocreaceae*	-0.207	0.043	0.622	-0.987	0.974	**0.000**	-0.787	0.620	**0.012**	-0.869	0.754	**0.011**
	Uncultured fungi vs *Chaetomiaceae*	-0.814	0.663	**0.014**	-0.825	0.680	**0.022**	-0.891	0.793	**0.001**	-0.714	0.510	**0.047**
	Uncultured fungi vs *Wallemiales incertae sedis*	0.343	0.118	0.406	-0.768	0.589	**0.016**	-0.844	0.713	**0.004**	-0.730	0.533	**0.040**
Between bacteria and fungi	*Bacillaceae vs Chaetomiaceae*	0.089	0.008	0.820	-0.708	0.501	0.116	-0.815	0.664	**0.007**	-0.528	0.279	0.144
	Bacillaceae vs Wallemiales incertae sedis	-0.378	0.143	0.316	-0.665	0.442	0.072	-0.904	0.818	**0.001**	-0.700	0.491	**0.036**
Positive													
Between bacteria	*Bacillaceae vs Nocardiopsaceae*	0.045	0.002	0.908	-0.258	0.066	0.503	0.700	0.490	**0.011**	0.586	0.344	**0.045**
Between Fungi	*Hypocreaceae vs* Uncultured fungi	0.369	0.136	0.416	0.844	0.713	**0.017**	0.795	0.632	**0.010**	0.637	0.405	0.124
Between bacteria and fungi	*Bacillaceae vs* Uncultured fungi	0.235	0.055	0.575	0.710	0.504	**0.049**	0.748	0.559	**0.021**	0.806	0.650	**0.016**
	Nocardiopsaceae vs Hypocreaceae	0.330	0.109	0.385	0.768	0.590	**0.016**	0.823	0.677	**0.006**	0.790	0.624	**0.011**
	Nocardiopsaceae vs Uncultured fungi	0.853	0.728	**0.007**	0.875	0.765	**0.002**	0.921	0.848	**0.000**	0.766	0.587	0.076

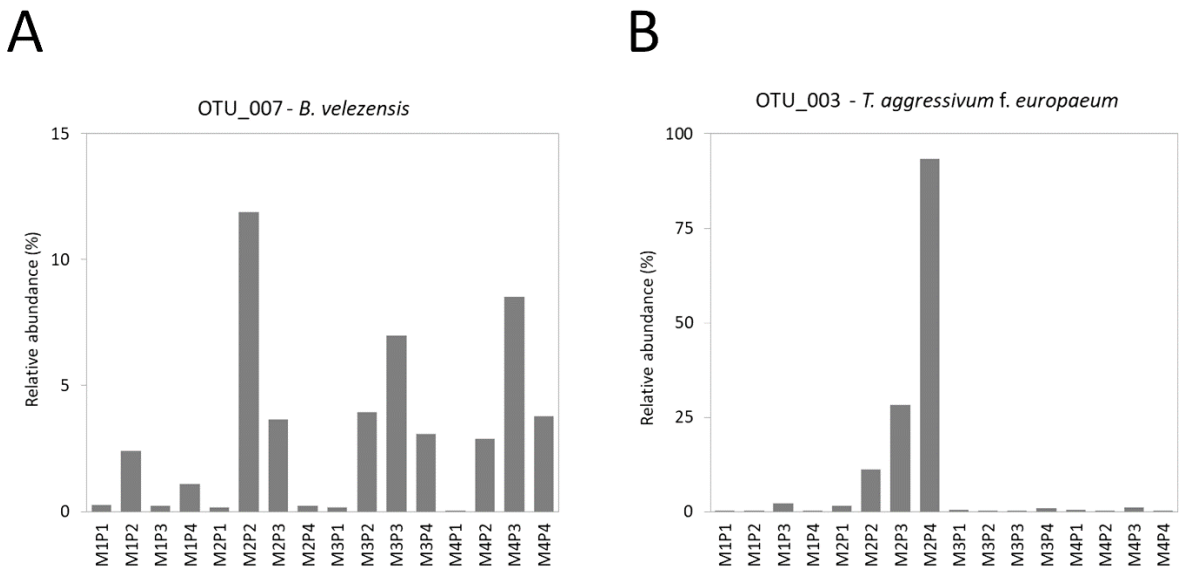

Fig. S1: Relative abundance of (A) OTU_007 (*B. velezensis*) and (B) OTU_003 (*T. aggressivum* f. *europaeum*) under all conditions over time.

Chapitre 3: Identification des gènes impliqués dans la formation de biofilm et la production d'antimicrobiens chez *Bacillus velezensis* QST713 dans le pathosystème

Préambule

Les données obtenues précédemment nous ont permis de réassigner l'agent de biocontrôle *B. subtilis* QST713 à l'espèce *B. velezensis*. Nous avons également déterminé en étudiant sa séquence génomique, que cette souche possédait tous les gènes nécessaires à la formation de biofilm, ainsi que les gènes nécessaires à la synthèse de nombreux métabolites secondaires potentiellement impliqués dans l'inhibition de *T. aggressivum*. Son aptitude à former des biofilms et inhiber le pathogène *T. aggressivum* a également été confirmée *in vitro*. L'étude de son impact sur les communautés microbiennes du compost de culture d'*A. bisporus*, réalisée au centre technique du champignon, a permis de déterminer qu'elle avait un impact modéré lorsqu'elle était présente dans le compost. Impact qui s'estompait avec la disparition de l'agent de biocontrôle jusqu'à une totale résilience du système en fin de culture. Nous avons également appris lors de ces expériences, que (i) cet effet de biocontrôle commençait avant le septième jour de culture, (ii) le compost était extrêmement riche en bactéries du genre *Bacillus* mais sans effet de biocontrôle de cette flore sur le pathogène *T. aggressivum*, faisant penser à un effet spécifique de la souche QST713.

Afin d'explorer l'hypothèse que la souche QST713 avait un effet de biocontrôle spécifique contre *T. aggressivum*, Nous avions tout d'abord opté pour la construction de mutants permettant l'inactivation de gènes impliqués dans la formation de biofilm et synthèse de métabolites secondaires. Cependant, la simple transformation de la souche QST713 avec un plasmide s'avérant extrêmement difficile (point abordé dans la discussion générale), nous avons opté pour une expérience permettant d'observer l'expression transcriptionnelles de ces gènes lors de la culture d'*Agaricus bisporus*. Dans ce contexte, nous avons développé au laboratoire, un micromodèle de culture en compost permettant la croissance d'*A. bisporus* en enceinte climatique lors de la première phase de culture de son cycle (quinze premiers jours), la phase végétative. De façon à observer spécifiquement la réponse de la souche QST713 à la présence du pathogène, nous avons décidé de travailler sur un modèle de culture simplifiée à trois partenaires (*B. velezensis* QST713, *A. bisporus* et *T. aggressivum*) en se soustrayant de la flore autochtone du compost. Nous avons, pour cela, fait subir au compost, une série de trois autoclavages entrecoupées de période de tyndallisation de 24h à 25°C permettant la germination des spores n'ayant pas été tuées lors de l'autoclavage précèdent. Une mise au point du pathosystème, en termes de concentration apportées de chaque partenaire, a été nécessaire afin de retrouver une vitesse de croissance d'*A. bisporus* équivalente au système complexe et un effet de biocontrôle similaire.

Grâce à ce micromodèle de culture axénique, nous nous sommes intéressés à l'état physiologique de la souche *B. velezensis* QST713 dans le compost lors de la culture d'*A. bisporus*. Forme-t-elle des biofilms sur ce substrat ? Nous avons également voulu déterminer par quels mécanismes, cet agent de biocontrôle permettait l'inhibition du compétiteur *T. aggressivum*. Les résultats de ce chapitre sont présentés sous forme d'un article (ci-après), en cours de finalisation : « Biofilm formation and antimicrobial synthesis by the biocontrol agent *Bacillus velezensis* QST713 in *Agaricus bisporus* compost micromodel ».

3.1 Article 5 : Biofilm formation and antimicrobial synthesis by the biocontrol agent *Bacillus velezensis* QST713 in *Agaricus bisporus* compost micromodel

Caroline Pandin[a], Maud Darsonval[a], Camille Mayeur[a], Dominique Le Coq[a,b], Stéphane Aymerich[a], Romain Briandet[a]

[a] Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[b] Micalis Institute, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78350 Jouy-en-Josas, France.

Article en cours de finalisation

Biofilm formation and antimicrobial synthesis by the biocontrol agent *Bacillus velezensis* QST713 in *Agaricus bisporus* compost micromodel.

Caroline Pandin[a], Maud Darsonval[a], Camille Mayeur[a], Dominique Le Coq[a,b], Stéphane Aymerich[a], Romain Briandet[a*]

[a]Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[b]Micalis Institute, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78350 Jouy-en-Josas, France.

*Corresponding author.
E-mail addresses: romain.briandet@inra.fr (R. Briandet)

ARTICLE INFO

Bacillus velezensis
Biofilm
qPCR
Biocontrol
Compost
Secondary metabolites
Gfp
CLSM

ABSTRACT

Bacillus velezensis QST713 is widely used as a biological control agent for crop protection and disease suppression. This strain is used industrially in France for the protection of *Agaricus bisporus* against *Trichoderma aggressivum* f. *europaeum*, the green mould disease agent. While the efficacy of this biocontrol process has been evaluated in a previous report, the mode of action has not yet been explored in production conditions. In order to decipher the underlying biocontrol mechanisms, we developed a simplified micromodel for the culture of *A. bisporus* during its early culture cycle. This micromodel allows to study the response of strain QST713 in axenic industrial compost in the presence or not of *A. bisporus* or/and *T. aggressivum*. We report a strong expression of several genes of the biocontrol agent involved in biofilm formation in the compost, along with surfactin and fengycin over-production in presence of the fungal pathogen.

1 Introduction

The strain *Bacillus velezensis* QST713 is a biocontrol agent used in France to protect *Agaricus bisporus* crop against *Trichoderma aggressivum*. Very recently, the genome of this strain has been published (Pandin et al., 2018a), as well as its impact on the microbial communities in the culture substrate of *A. bisporus* and its biocontrol effect on *T. aggressivum* (Pandin et al., 2018b). Many studies invoked biofilm formation and antimicrobial production in bioprotective mechanisms of crop by *Bacillus* species (Khezri et al., 2011; Xu et al., 2014; Zeriouh et al., 2014; Aleti et al., 2016;

Pandin et al., 2017). To determine the involvement of biofilm formation and *B. velezensis* antimicrobial synthesis in the bioprotective mechanisms during the culture of *A. bisporus*, an immersion in the well documented regulation of *B. subtilis* biofilm formation is first necessary. The transition from the "planktonic" to the "biofilm" state requires regulatory pathways to control the expression of genes involved in matrix production or motility. Indeed, one of the characteristics of biofilms is their cellular heterogeneity (Vlamakis et al., 2008, 2013). This cellular heterogeneity is illustrated by the coexistence of different cell types such as matrix-producing cells, surfactin-producing cells, flagellated cells or sporulated cells. Each cell type is characterized by differential expression of specific genes regulated by genetic determinants involved in various regulatory pathways for biofilm formation (Vlamakis et al., 2008; Mielich-Süss and Lopez, 2015; van Gestel et al., 2015).

Table 1: Strains, plasmid and primers used in this study.

Strains	**Description**	**Source**
Escherichia coli		
GM48	F-, *hr-1*, *araC14*, *leuB6*(Am), *fhuA31*, *lacY1*, *tsx-78*, *glnX44*(AS), *galK2*(Oc), *galT22*, λ^-, *dcm-6*, *dam-3*, *thiE1*	Lab strain
Bacillus velezensis		
QST713	Wild type	Serenade (Bayer)
QST713-*gfp*	carrying pHAPII plasmid, Km^r	This study
SQR9-*gfp*	carrying pHAPII plasmid, Km^r	Cao et al. 2011
FZB42-FB01	*amyE*::Em^r-*gfp*+	Fan et al. 2011
Trichoderma aggressivum f. *europaeum* (Ta2)		
strain Z	Wild type	Mamoun et al. 2000
Agaricus bisporus		
IE-751 (Delta)	Wild type	Amycel
Plasmid		
pHAPII	pUBC19 containing *gfp* fused to HAPII promoter	Cao et al. 2011
Primers for *B. velezensis* QST713	**Oligonucleotide sequence 5' – 3'**	
ForblsA	AATTTTTCTCAACTGTCATGGCAAG	This study
RevblsA	CGTTGACTGTATCTTTTGTTGTAGC	This study
ForbmyA	GAGCTCTGTGACGAATTTTTGAA	This study
RevbmyA	CTACTCTTTCATTTGTTTCATCGGA	This study
FordhbA	CGTAAATGTGGCAGCTATAGATA	This study
RevdhbA	GCAACGTTCGCCAATATTTCAAT	This study
ForepsC	TCAGGCTATACGACAGAACAGAT	This study
RevepsC	AATCTGCTCCGTATGTACTTCA	This study
ForfenA	CAGAAACGGATGTATGTGCTC	This study
RevfenA	GCTTCCTCCAGACCAAACG	This study
Forhag	ATATCGCGGCTCTTAACACTAG	This study
Revhag	ATACGCTGAAGAATGCTGTGA	This study
FornrpsB	TTTGGTGGATGTTAGCGGGTGGTA	This study
RevnrpsB	TGTAGTTTCTGCTGGACCATAG	This study
ForsrfAA	CCTGTCCCGTGTGATCAAAC	This study
RevsrfAA	GACTTCCGTATTGCTGACCG	This study
ForswrA	GTGGAACAACTAAAAGACAGAACA	This study
RevswrA	TCAGATAGTGGTCAACCTCC	This study
FortapA	CAAGGTGAGATGGCGGATGA	This study
RevtapA	GCTCTTTTCCCTTATTCTCCAG	This study
FortnrpsC	ACTGGAGAATACGGTGGGGA	This study
RevtnrpsC	CAAGTACAAACATGACATCAAACA	This study
ForgyrA	CAGGTGAACAAAGCGAGATTAATT	This study
RevgyrA	GTGATCAAGATAATGCTCCAGACA	This study

The activation of these different regulatory pathways goes through the recognition of extracellular signals (presence of biocides, surfactants, plant exudates, etc.), that is orchestrated by the KinA, KinB, KinC and KinD kinase phosphatases, which allow the phosphorylation of the Spo0A regulator and thus activates the biofilm formation (Vlamakis et al., 2013).

The transcriptional regulator Spo0A controls the expression of about hundred genes, including the genes encoding the transcriptional regulators AbrB, SinI/SinR, SlrA/SlrR, triggering the direct or indirect regulation of genes involved in the synthesis of extracellular matrix components (*tasA* operon, *eps* operon) or motility (*hag*) (Vlamakis et al., 2013). The *tasA* operon (*tapA-sipW-tasA*) allows the synthesis of the TasA structural protein present in the matrix forming amyloid fibers. The *eps* operon (*epsA-O*) is involved in the production of exopolysaccharides present in the matrix (Branda et al., 2006; Romero et al., 2010; Vlamakis et al., 2013). The *hag* gene encodes a flagellar protein expressed in motile cells (Vlamakis et al., 2013). Spo0A is involved in the regulation of *srf* genes allowing the synthesis of surfactin, a surface-active lipopeptide involved in biofilm formation by induction of polymer synthesis and involved in the regulation of *swr* genes of the swarming motility system (Kearns et al., 2004). Surfactin can act as a signaling molecule and promote the formation of biofilms from other Bacilli, but also as a wide spectrum antimicrobial (López et al., 2009; Aleti et al., 2016). Spo0A also allows the regulation of the *sigF* gene involved in sporulation (Vlamakis et al., 2013; Mielich-Süss and Lopez, 2015; van Gestel et al., 2015).

Another regulator, DegU, is also involved in the regulation of the genes of the matrix including (i) *blsA* that encodes an amphiphilic hydrophobin (BlsA) forming a hydrophobic protective layer on the interface of the biofilm in contact with air and (ii) the *pgsBCD* operon that allows the synthesis of a polymer giving a mucosal appearance to the colonies. DegU is also involved in antibiotic production, and it controls the expression of *swr* genes involved in swarming motility (Kearns et al., 2004; Verhamme et al., 2007; Vlamakis et al., 2013; Xu et al., 2014; Mielich-Süss and Lopez, 2015). The ComA regulator also controls the production of surfactin, as well as the ability to incorporate exogenous DNA from the environment (López and Kolter, 2010; Mielich-Süss and Lopez, 2015; van Gestel et al., 2015).

It has been also shown that certain antimicrobials can be overproduced in biofilm lifestyle (Kröber et al., 2016), thereby conferring an additional advantage on the protection provided by the biofilm mode of life. We hypothesized that both the biofilm-associated spatial competition and the antifungal production by *B. velezensis* QST713 could be both involved in the bioprotective mechanisms against *T. aggressivum* during the cultivation of *A. bisporus*. In this contribution, we studied the expression of genes related to biofilm formation and antimicrobial production of *B. velezensis* QST713 according to the study of its genomic sequence (Pandin et al., 2018a); (i) in broth compost extract for exponential and stationary growth phase, and (ii) in a culture compost with or without the presence of *Trichoderma aggressivum* f. *europaeum* and *Agaricus bisporus*. The culture compost of *A. bisporus* is naturally extremely rich in *Bacillus* species (Pandin et al., 2018b). Therefore, we chose to carry out this study on autoclaved axenic compost in order to specifically study the cell reprogramming of strain QST713. We also studies the spatial organization of the biocontrol agent in the compost by taking advantage of Gfp expressing *B. velezensis* strains combined with with non-invasive confocal laser scanning microscopy (CLSM).

2 Materials and Methods

2.1 Strains and culture conditions

The strains and plasmid used in this study are described in Table 1. *Bacillus velezensis* QST713 (Pandin et al., 2018a), *Bacillus velezensis* SQR9-*gfp* (Cao et al., 2011) and *Bacillus velezensis* FZB42-FB01 were used in this study. *Escherichia coli* GM48 was used as the host strain for the

plasmid pHAPII. *Bacillus* strains were cultured in Trypticase Soy Broth (TSB, Biomerieux, France) (1% tryptone, 0.5% yeast extract, 0.5% NaCl) 24h at 25°C, 200 rpm. Antibiotics were added as required at the following concentrations: 25 µg mL^{-1} kanamycin (Kan), 1 µg mL^{-1} erythromycin (Em). *E. coli* GM48 cells were grown in LB medium 24h at 37°C, 200 rpm. When necessary, 25 µg mL^{-1} of kanamycin was added. *Trichoderma aggressivum* f. *europaeum* Ta2 strain Z (Mamoun et al., 2000; Largeteau and Savoie, 2010) and *Agaricus bisporus* (Amycel Delta, white hybrid variety, Amycel, Vendôme) were cultured on YMEA medium (2 $g.L^{-1}$ yeast extract (Sigma-Aldrich), 20 $g.L^{-1}$malt extract (Sigma-Aldrich), 15 $g.L^{-1}$Agar (Sigma-Aldrich)) supplemented with a mixture of citric acid (250 $\mu g.ml^{-1}$) (Sigma-Aldrich), streptomycin (100 $\mu g.ml^{-1}$) (Sigma-Aldrich), and tetracycline (50 $\mu g.ml^{-1}$) (Sigma-Aldrich) for 15 days at 25°C. Cubes of *A. bisporus* YMEA cultures were used to produce an initial culture of *A. bisporus* in autoclaved compost and incubated in a climatic chamber for 15 days (25°C, hygrometry 90%).

2.2 Preparation of axenic compost and broth compost extract.

The industrial compost used in this experiment is composed of straw and horse manure, supplemented with nitrogen (urea and ammonium sulphate), minerals (gypsum and calcium carbonate), and water (France Champignon, La Tourte, Longué-Jumelles, France). For the preparation of autoclaved axenic compost, 50 g of fresh compost was deposited into individual pots (Microbox Full Gas 560 mL 97x80 mm XXL filter lid, Combiness) and autoclaved three times (30 min; 121°C) spaced by 24 hours at 25°C before experiment and stored at 4°C. For the preparation of broth compost extract, 300 g of fresh compost was cooked in 1 L of water for 20 min, then centrifuged; the supernatant was collected and supplemented with 5 g of anhydrous glucose; the mixture was autoclaved for 30 min at 121°C then filtered with Stericup-GP-0.22 µm (Merck-Millipore).

2.3 Broth compost extract experiment.

B. velezensis QST713 was cultivated in broth compost extract 24h at 25°C, 200 rpm. Optical density at 600 nm (OD_{600}) was measured, serial dilution and enumeration were made to determine the growth curve of the strain and perform RNA extraction on samples collected during the exponential and stationary growth phases. For the broth compost extract samples, 4 mL of culture was collected in mid-exponential growth phases and 2 mL in late stationary growth phase then centrifuged, supernatant was removed and 1 mL of Lifeguard solution (Qiagen) was added. The six samples were stored at -20°C (performed in triplicate) until their RNA extraction.

2.4 Culture compost axenic micromodel experiments.

Cultures in compost were performed with treatment with *B. velezensis* QST713 with (A. b +) or without (A. b -) seeding of *A. bisporus*, and with (T. a +) or without (T. a -) inoculation of *T. aggressivum*. Hence, four conditions were studied: strain QST713 alone in the compost (B: QST713 +; A. b -; T. a -), strain QST713 with *T. aggressivum* in the compost (BT: QST713 +; A. b -; T. a +), strain QST713 with *A. bisporus* in the compost (BA: QST713 +; A. b +; T. a -), and strain QST713 with both in the compost (BTA: QST713 +; A. b +; T. a +). For all conditions, individual pots of 50 g of autoclaved compost were used, 5 mL *Bacillus velezensis* QST713 suspension in physiological water, at 50 CFU mL^{-1}, was added into each pot and final concentration of strain QST713 was 2.5 x 10^2 CFU per pot. When required, the compost was then seeded with 1.6 g of a 15 days-old culture of *A. bisporus* in autoclaved compost, and/or inoculated with 5 mL *T. aggressivum* spores solution in physiological water at 200 spores mL^{-1} made with conidia collected from a 15 days-old YMEA culture. *T. aggressivum* was set at a final concentration of 1000 spores per pot. When required, 5 mL of physiological water was added in the pots to equilibrate the water supply. The compost was then homogenized and compacted at the bottom of the pot. Three replicates per condition were

performed representing twelve pots in total. The pots were then incubated under controlled conditions during 10 days of culture in climatic chamber (25°C, hygrometry 90%). Samples were taken on the 6th day of culture. For each compost sample (twelve in total), a weight of 5 g of compost was collected and crushed with mortar and pestle in liquid nitrogen for homogenization. Samples were finally stored at -80°C until their RNA extraction.

2.5 *Bacillu*s extraction and enumeration from the compost.

For each pot, compost was collected at 0, 3, 6 and 10 days. Strain QST713 extraction was performed from 5 g compost in 45 mL sterile physiological saline water (0.85% NaCl) with a sterile filter bag Interscience Bag System ® (Interscience, Saint-Nom, France). The mixture was homogenized for 30 min at room temperature and then blended in a commercial laboratory blender according to the manufacturer's instructions (Seward Laboratory Lab-Blender 400, Worthing, UK). The filtered liquid was collected and readjusted to 50 ml with sterile physiological saline water. Serial dilutions were made from the extracted suspensions in sterile physiological saline water (0.85% NaCl) and 0.1 mL of each serial dilution was used to inoculate Trypticase Soy Agar (TSA; Biomerieux, France) in a Petri dish. All Petri dishes were incubated 48h at 25°C.

2.6 RNA extraction method.

RNA extraction of strain QST713 from compost was performed with the Lysozyme / CTAB (hexadecyltrimethylammonium bromide) / TriReagent (Sigma-Aldrich) / Chloroforme method with some modifications (Jordon-Thaden et al., 2015). The CTAB extraction buffer was prepared according to Wang and Stegemann (2010) with some modifications and was composed of 2% CTAB (Sigma-Aldrich), 2.5% polyvinylpyrrolidone (PVP 40; Sigma-Aldrich), 2 M sodium chloride (NaCl; Sigma-Aldrich), 100 mM Tris-HCl (pH8; Sigma-Aldrich), 20 mM ethylene-diamine-tetra-acetic acid (EDTA; Sigma-Aldrich) and 2% beta-mercaptoethanol (added just before use) (Sigma-Aldrich) in RNase free water (Merck – Millipore) at a final volume of 100 mL. Before the RNA extraction, the broth compost extract culture samples were centrifuged to remove the LifeGuard solution and 100 mg of each homogenized compost samples were collected. A lysozyme treatment for 30 min at 37°C in 500 µL of lysozyme buffer (30 mM Tris, 10mM EDTA, 10 mg mL^{-1} lysozyme (Sigma-Aldrich), pH 6.2 in RNase free water) was performed on the eighteen samples. After the lysozyme treatment, 500 µl of CTAB buffer supplemented with proteinase K at 20 mg mL^{-1} final concentration was added, vortexed for 2 min and incubated in a water bath at 55°C for 10 min. The next steps of the RNA extraction were carried out according to appendix 5, step 30 to 56 of Jordon-Thaden et al. (2015), followed by DNase treatment in solution with the RNase-Free DNase I Kit (Norgen Bioteck) and RNA Clean-Up Kit (Norgen bioteck). Presence of RNA was verified with NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific) and Qubit 3.0 Fluorometer (ThermoFisher Scientific). Quality and concentration of RNA was assessed by Bioanalyzer Agilent 2100 (Agilent Technologies) at the @BRIDge platform (http://abridge.inra.fr/). When required, RNA were precipitated with glycogen (Glycogen, molecular biology grade; ThermoFisher Scientific) according to manufacturer protocol to obtain 1 µg of total RNA required for reverse transcription.

2.7 Gene transcription analyses by qPCR.

The reverse transcription of RNA was conducted with the Omniscript Reverse Transcription kit (Qiagen) according to manufacturer protocol with random hexamers (Qiagen). Reverse transcripts of the genes involved in biofilm formation, antimicrobial synthesis and motility (*epsC*, *tapA*, *blsA*, *srfAA*, *fenA*, *bmyA*, *dhbA*, *nrpsB*, *tnrpsC*, *hag* and *swrA*) were quantified by qPCR with the Takyon™ Rox SYBR® MasterMix dTTP Blue (Eurogentec) according to manufacturer protocol (10 µL of Takyon ™ MasterMix, 0.4 µL of each primer

(final concentration 200 nM), 5 µL of cDNA template diluted 20X, 4.2 µL of water for molecular biology). The qPCR protocol for maximal sensitivity was performed, 5 min at 95°C then followed by 40 cycles of denaturation 15 s at 95°c, annealing 1 min at 60°C, elongation 30 s at 72°C. A final elongation was performed 5min at 72°C followed by a melt curve stage. The *gyrA* gene was used as an internal control (Table 1). The $2^{-\Delta\Delta CT}$ method was used to analyse the qPCR data and results were presented as Log2(fold change) (Livak and Schmittgen, 2001).

2.8 *Bacillus velezensis* QST713 transformation.

In order to fluorescently contrast the strain QST 713 in the compost by CLSM, it was necessary to transform it to allow the expression of the Gfp (green fluorescent protein). However, transformation of strain QST713 is very difficult and, despite many attempts, has not be reported elsewhere. We tested many methods and only one succeed, with a very low efficacy. The method used here combines methods described in Zhang et al. (2011) and Yi and Kuipers (2017), with some modifications. It is based on the use of unmethylated plasmid, a growth medium with high osmotic pressure and the use of glycine to weaken the peptidoglycan. Prior to transformation, plasmid pHAPII (carrying genes coding a Gfp) was extracted from strain SQR9-*gfp* with the GeneJET Plasmid Miniprep Kit (ThermoFisher). Transformation of *Escherichia coli* GM48 (*dam*$^-$/*dcm*$^-$) was performed to demethylate the pHAPII plasmid and plasmid extraction was realized. For the transformation of strain QST713, competent cells were prepared according to the following protocol. Cells were grown in 50 mL BHIS medium (34 g BHI (Difco), and 91.1 g sorbitol in 1 L deionized water, pH 7.2) at 30°C, 200 rpm to OD_{600} = 0.85. After 1 h of additional incubation with 1% glycine, the cell culture was transferred into a 50 mL centrifuge tube and cooled on ice for 20 min, and cells were collected (OD_{600} = 0.95) by centrifugation at 4 °C, 8000g for 5 min. Cells were washed four times in electroporation buffer (pre-chilled) (10% glycerol, 0.25 M sorbitol, 0.25 M mannitol) and electroporation buffer was added to the pellet at 1/100 vol of the original culture to obtain electro-competent cells. Electroporation was performed from 100 µL of electro-competent cells with 250 ng of pHAPII plasmid in 2-mm gap electroporation cuvettes (pre-chilled) with the Eppendorf Eporator ® (strength 2,1 kV cm^{-1}) (Eppendorf). After pulse, 1 mL of pre-warmed growth medium was added and transferred to a 2 mL eppendorf tube and incubated for a recovery time of 5h at 30°C, 180 rpm.

Electroporation was centrifuged, supernatant was removed and 100 µL of sterile growth medium was added and the total resulting volume was plated on LB agar plates containing 25 µg mL^{-1} of kanamycin. Transformation was verified by visualisation of colony fluorescence on a blue light table.

2.9 Confocal Laser Scanning Microscopy of Gfp-Bacilli in axenic compost.

The condition *Bacillus*/*Trichoderma*/*Agaricus* in compost described above was used to visualize the biocontrol agent spatial organization at 6 days in the compost by confocal laser scanning microscopy (CLSM), using a Leica SP8 AOBS inverter confocal laser scanning microscope (Leica, Microsystems, Germany). The autofluorescent bacteria used were *Bacillus velezensis* QST713 carrying a plasmid encoding a Gfp (this study), and two relative biocontrol strains *Bacillus velezensis* SQR9 carrying a plasmid encoding a Gfp (Cao et al., 2011) or *Bacillus velezensis* FZB42-FB01 Gfp tagged (Fan et al., 2011). Samples were collected in pots and directly scanned using 10x and 20x HC PL FLUOTAR dry objective lens. Samples were scanned at 600 Hz (1024 x 1024 pixels images, z step of 4 µm, z range from 480 to 900 µm), with dual excitation wavelengths of 488 nm (argon laser: intensity 44%) and 561 nm (DPSS 561 laser; intensity 1.88%). Using ultra-sensitive hybrid detectors (HyD Leica Microsystems, Germany), the emitted fluorescences were collected in the range 497 to 533 nm for the green Gfp (bacilli) and in the range 580 to 740 nm for the compost red auto-fluorescence. Laser

reflexion was used simultaneously to contrast the mycelium of *A. bisporus*. Three-dimensional projections from the *xyz* image series were reconstructed using the Easy 3D function of the IMARIS software (Bitplane, Switzerland).

Fig. 1: Growth of *A. bisporus* and *T. aggressivum* in culture micromodels and effect of *B. velezensis* QST713 in co-culture with one or both partners during 15 days of culture. Pictures of culture pots were taken at six (6d), ten (10d) and fifteen days (15d) of culture. From the top line to the bottom line: (line 1: QST713 -; A. b +; T. a -) *A. bisporus* alone in the compost resulting in the progressive invasion of the compost by a white mycelium; (line 2: QST713 -; A. b -; T. a +) The development of *T. aggressivum* alone in the compost with a massive sporulation (mostly at six days); (line 3: QST713 -; A. b +; T. a +) Pathogenic effect of *T. aggressivum* on *A. bisporus*, absence of *A. bisporus* mycelium development and massive sporulation of *T. aggressivum* (mostly at 6 days); (line 4: QST713 +; A. b -; T. a -) *B. velezensis* QST713 alone in the compost, compost has a natural aspect; (line 5: QST713 +; A. b -; T. a +) the co-culture of *B. velezensis* QST713 and *T. aggressivum*, absence of development of *T. aggressivum* that showed the inhibitory effect on T. aggressivum by strain QST713, and natural aspect of the compost; (line 6: QST713 +; A. b +; T. a -) the co-culture of *B. velezensis* QST713 and *A. bisporus*, normal development of *A. bisporus* mycelium; (line 7: QST713 +; A. b +; T. a +) the co-culture of *B. velezensis* QST713 with *T. aggressivum* and *A. bisporus*, normal development of *A. bisporus* mycelium that showed the biocontrol effect of *B. velezensis* QST713 on *T. aggressivum*.

2.10 Statistical analysis.

The Shapiro-Wilk and Levene tests were applied to determine the normality of the data distribution and homogeneity of variances. The data were not normally distributed and the variances were not homogeneous. Thus, a non-parametric Kruskal-Wallis ANOVA test and the Conover-Iman post-hoc test were performed to determine whether the observed differences were significant using XLSTAT-Premium trial software (Microsoft Corporation, USA).

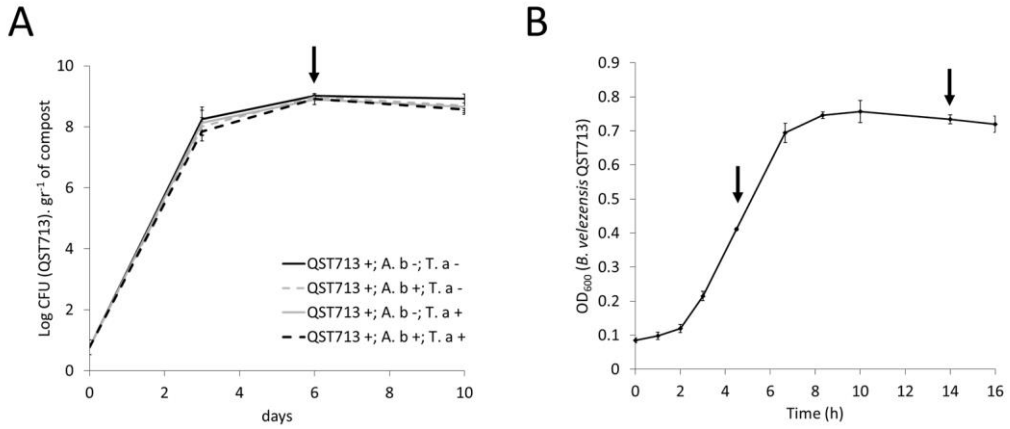

Fig. 2: Growth of *B. velezensis* QST713 in the compost and broth compost extract. (A) Enumeration of strain QST713 (QST713+) cultivated in the compost micromodels during 10 days of culture at 25°C in climatic chamber with (A. b +) or without (A. b -) *A. bisporus* and with (T. a +) or without (T. a -) *T. aggressivum*. (B) Growth curves of *B. velezensis* QST713 in broth compost extract with the measurement of the optical density of the culture at 600 nm (OD_{600}) for 16 hours at 25°C, 200 rpm. Three replicates (n=3) were performed for both experiments. Bars represent the standard deviation. Arrows represent the time points where the cells were taken for the RNA extraction experiment.

3 Results

3.1 Growth and biocontrol effect of *B. velezensis* QST713 in compost micromodels and broth compost extract.

The validation of *A. bisporus* culture micromodels in axenic autoclaved compost was based on the invasion of compost by the mycelium of *A. bisporus* at the end of the vegetative phase (15 days of culture), as well as the observation of the biocontrol effect of *B. velezensis* QST713 on *T. aggressivum*. Three controls were carried out: (1) the normal development of *A. bisporus* alone in the compost resulting in the progressive invasion of the compost in pots by a white mycelium viewable on the first line in Fig. 1; (2) The development of the competitor *T. aggressivum* alone in the compost with a massive sporulation at six days (line 2, Fig. 1); (3) The pathogenic effect of *T. aggressivum* on *A. bisporus*, resulting in the absence of *A. bisporus* mycelium development during the culture and the colonisation of *T. aggressivum* that was the most observable at 6 days (line 3; Fig. 1). The four conditions studied are shown in the Fig. 1 (lines 4, 5, 6 and 7). Presence of strain QST713 alone (line 4; Fig. 1) did not affect the compost natural appearance. In the second condition characterized by the co-culture of strain QST713 and *T. aggressivum*, we observed the absence of *T. aggressivum* sporulation in a healthy compost showing the inhibition effect of *B. velezensis* QST713 on *T. aggressivum* (line 5; Fig. 1). In the third condition assessed to determine whether the development of *A. bisporus* was disturbed, we observed a normal growth of *A. bisporus* in the presence of the biocontrol agent *B. velezensis* QST713 (line 6; Fig.1). The last condition studied was the co-culture of *A. bisporus*/*T. aggressivum*/*B. velezensis* QST713 to validate the biocontrol

efficiency of strain QST713; we observed a normal growth of the mycelium of *A. bisporus* and an inhibition of the development of *T. aggressivum* (line 7; Fig.1). Enumeration of *B. velezensis* QST713 was assessed to determine its effective development in the compost and results are represented in Fig. 2A. Strain QST713 alone in compost reached 10^9 CFU g^{-1} of compost at 6 days of culture and its development was not affected by the presence of the fungi ($P > 0.05$).

In order to carry out the RNA extraction from planktonic cells in exponential and stationary growth phase in the liquid broth compost extract, the growth curve of *B. velezensis* QST713 was determined. Strain QST713 reached the stationary growth phase in the broth compost extract at eight hours of culture at 25°C (200 rpm) (Fig. 2B). Optical density at 600 nm was 0.74, corresponding to 4 x 10^7 CFU mL^{-1}. Exponential growth phase occurred from three to six hours. Cells were collected in the mid exponential growth phase at 5 h of culture and in stationary growth phase at 14 h of culture.

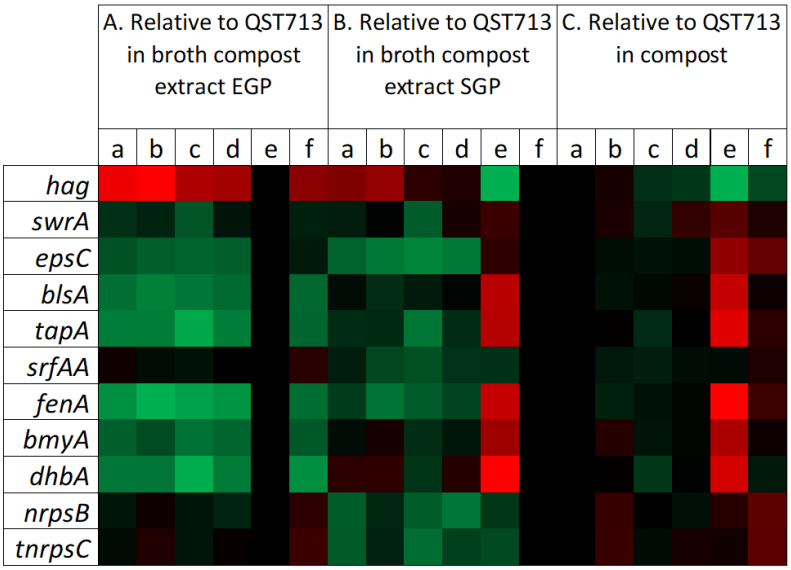

Fig. 3: Heatmap of the transcriptional levels of biofilm-related, secondary metabolites production and motility genes of *B. velezensis* QST713 in the compost and broth compost extract. Transcriptional levels of motility related genes (*hag* and *swrA*), biofilm-related genes (*epsC*, *blsA*, *tapA*, *srfAA*) and secondary metabolites synthesis genes (*fenA*, *bmyA*, *dhbA*, *nrpsB*, *tnrpsC*) for six different conditions: (a) *B. velezensis* QST713 alone in the compost, (b) the co-culture of *B. velezensis* QST713 and *T. aggressivum*, (c) the co-culture of *B. velezensis* QST713 and *A. bisporus*, and (d) the co-culture of *B. velezensis* QST713 with *T. aggressivum* and *A. bisporus*, (e) Exponential growth phase (EGP) of *B. velezensis* QST713 alone in the broth compost extract and (f) Stationary growth phase (SGP) of *B. velezensis* QST713 alone in the broth compost extract. Letters A, B and C represent the transcription level of QST713 genes relative to EGP, SGP or QST713 alone in the compost respectively. The green colour represents the values of up-regulated genes (max value: +7.19), the red represents the values of down-regulated genes (min value: -7.49), both compared to a control value of 0 (black). Data are represented in Log2Fold change.

3.2 Biofilm is the preferential mode of life of *B. velezensis* QST713 in compost micromodels.

To determine the state of *B. velezensis* QST713 in compost, we compared the differential expression of genes related to biofilm formation of strain QST713 cultivated in broth compost extract as a control of planktonic state at two different times (Exponential Growth Phase = EGP; Stationary Growth Phase = SGP), and in compost at six days of culture. Expression of

genes of strain QST713 in the compost relative to its growth in the broth compost extract at EGP and SGP, were represented as a heatmap (Fig. 3(Aa); (Ba)). Genes related to biofilm formation (*epsC*, involved in the synthesis of extracellular polysaccharide, *blsA*, involved in the production of the hydrophobin BlsA, and *tapA*, involved in the production of amyloid fibres) were up-regulated in the compost compared to EGP (Fig. 4A).

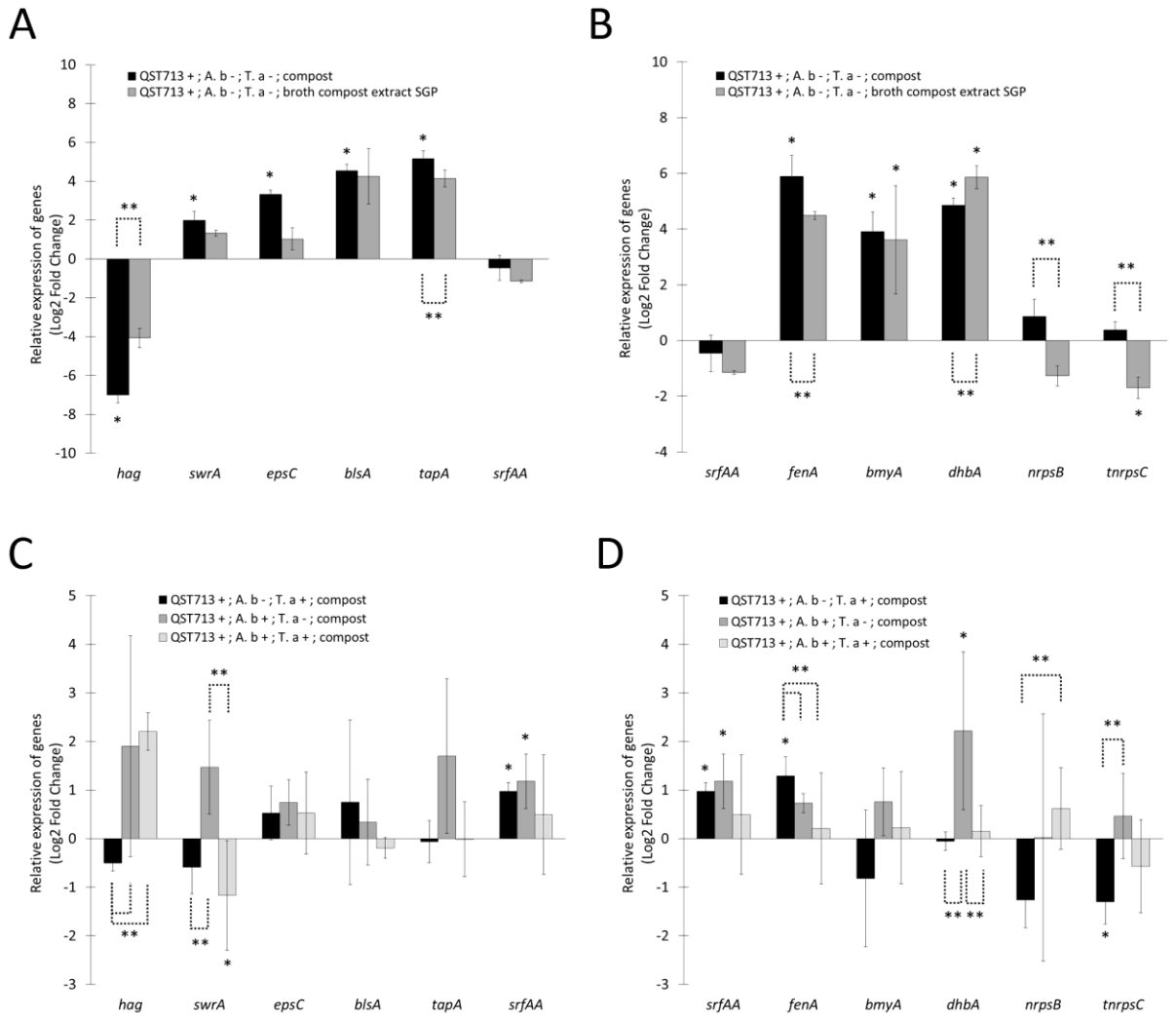

Fig. 4: Compost and interaction effects on the expression of genes related to motility, biofilm formation and secondary metabolites production in *B. velezensis* QST713. Transcriptional levels of motility-related genes (*hag* and *swrA*), biofilm-related genes (*epsC*, *blsA*, *tapA*, *srfAA*) and secondary metabolites synthesis genes (*fenA*, *bmyA*, *dhbA*, *nrpsB*, *tnrpsC*) relative to that of (A and B) the exponential growth phase of strain QST713 alone in the broth compost extract, and of (C and D) strain QST713 alone in the compost. Different culture conditions were studied: strain QST713 alone in the compost (QST713 +; A. b -; T. a -; compost), co-cultured with *T. aggressivum* in the compost (QST713 +; A. b -; T. a +; compost), co-cultured with *A. bisporus* in the compost (QST713 +; A. b +; T. a -; compost), co-cultured with *T. aggressivum* and *A. bisporus* (QST713 +; A. b +; T. a +; compost), and its stationary growth phase alone in the broth compost extract (QST713 +; A. b -; T. a -; broth compost extract SGP). The gene *gyrA* was used as an internal reference gene. Bars represent standard deviations of data from three biological replicates. Results are presented as Log2Fold Change with Fold Change = $2^{-\Delta\Delta CT}$. (*) represents the significant difference compared the exponential growth phase for A and B, and to strain QST713 alone in the compost for C and D ($P < 0.05$). (**) represents the significant difference between conditions ($P < 0.05$).

On the opposite, *hag*, a gene involved in flagellin biosynthesis was strongly down-regulated in the compost as compared to both EGP and SGP (Fig. 4A). The gene *swrA*, involved in swarming motility, was up-regulated in the compost compared to EGP. All together, these results strongly suggest that biofilm is the dominant mode of life of the strain QST713 in the compost.

3.3 What about *B. velezensis* QST713 secondary metabolites synthesis in axenic compost micromodel?

Expression of genes involved in the production of secondary metabolites in strain QST713 were studied for the planktonic lifestyle (broth compost extract) compared to compost-associated mode of life (compost) (Fig. 3 (A,a); (B,a)). Expression of the gene encoding surfactin, a molecule involved in biofilm formation and with antimicrobial activity, was not differently expressed between the biofilm (compost) and the planktonic lifestyle (EGP and SGP) (Fig. 4B). Three genes encoding secondary metabolites were up-regultated in biofilm state compared to EGP: *fenA*, involved in the synthesis of the antifungal fengycin, *bmyA*, involved in the synthesis of the antifungal bacillomycin D, and *dhbA*, involved in the synthesis of the siderophore bacillibactin. Only the gene *fenA* was up-regulated in the compost as compared to both EGP and SGP (Fig. 4B), which suggested that *fenA* was up-regulated in the compost biofilm mode of life. The genes *nrpsB* and *tnrpsC* of unknown functions, were neither differentially expressed in the planktonic state nor in the compost.

3.4 Antifungal compounds surfactin and fengycin are sur-expressed in the presence of *T. aggressivum*.

To determine the genes potentially involved in the bioprotection of *A. bisporus* culture by *B. velezensis* QST713 against *T. aggressivum*, expression of genes involved in biofilm formation and secondary metabolites production were studied in compost micromodels. The four conditions studied were, (a) *B. velezensis* QST713 alone in the compost (QST713 +; A. b -; T. a -), (b) the co-culture of *B. velezensis* QST713 and *T. aggressivum* (QST713 +; A. b -; T. a +), (c) the co-culture of *B. velezensis* QST713 and *A. bisporus* (QST713 +; A. b +; T. a -), and (d) the co-culture of *B. velezensis* QST713 with *T. aggressivum* and *A. bisporus* (QST713 +; A. b +; T. a +). Expression of genes of strain QST713 in the presence of *T. aggressivum*, *A. bisporus* or both in the compost were compared to those of strain QST713 alone in the compost and represented in Fig. 3 (C. a, b, c, d). The genes related to biofilm formation did not appear to be significantly up-regulated or down-regulated in the presence of *T. aggressivum*, *A. bisporus* or both compared to strain QST713 alone in the compost (Fig. 4C), which would be due to the huge variability between the biological replicates and a small sample size for an environmental experiment (n=3). The gene *srfAA* involved in the synthesis of the surfactin, molecule involved in the biofilm formation initiation and with an antifungal activity, was up-regulated in the presence of *T. aggressivum* or *A. bisporus* compared to strain QST713 alone in the compost. Another gene encoding an antifungal compound, *fenA*, was up-regulated in the presence of *T. aggressivum* compared to strain QST713 alone in the compost (Fig. 4D). The gene of unknown function, *tnrpsC*, encoding a potential secondary metabolite compound, was down-regulated in the presence of *T. aggressivum*. The gene *dhbA*, encoding the siderophore bacillibactin, was up-regulated in the presence of *A. bisporus* compared to strain QST713 alone in the compost (Fig. 4D). An iron competition likely occurs in the compost between strain QST713 and *A. bisporus*, effect that did not occur in the presence of the three partners.

3.5 Direct visualisation of biofilm formation in the compost by fluorescent *B. velezensis* strains.

Non-invasive confocal laser scanning microscopy experiment was performed for the condition where the three partners were present to confirm the effective biofilm formation by strain

QST713 on compost micromodels. The plasmid pHAPII (carrying genes encoding a Gfp) was electroporated in strain QST713. The transformation of the recalcitrant strain QST713 remains complicated and difficult to reproduce. However, strain QST713/pHAPII permitted to visualize the spatial organization of these bacteria in the compost at six days of culture (Fig. 5A). It was mainly observed in compost that strain QST713 formed micro-aggregates of cells (Fig. 5A, left picture) and sometime massive spatially organized biofilms (Fig. 5A, right picture). Systematic proximity of strain QST713 with *A. bisporus* mycelium was not observed during this experiment.

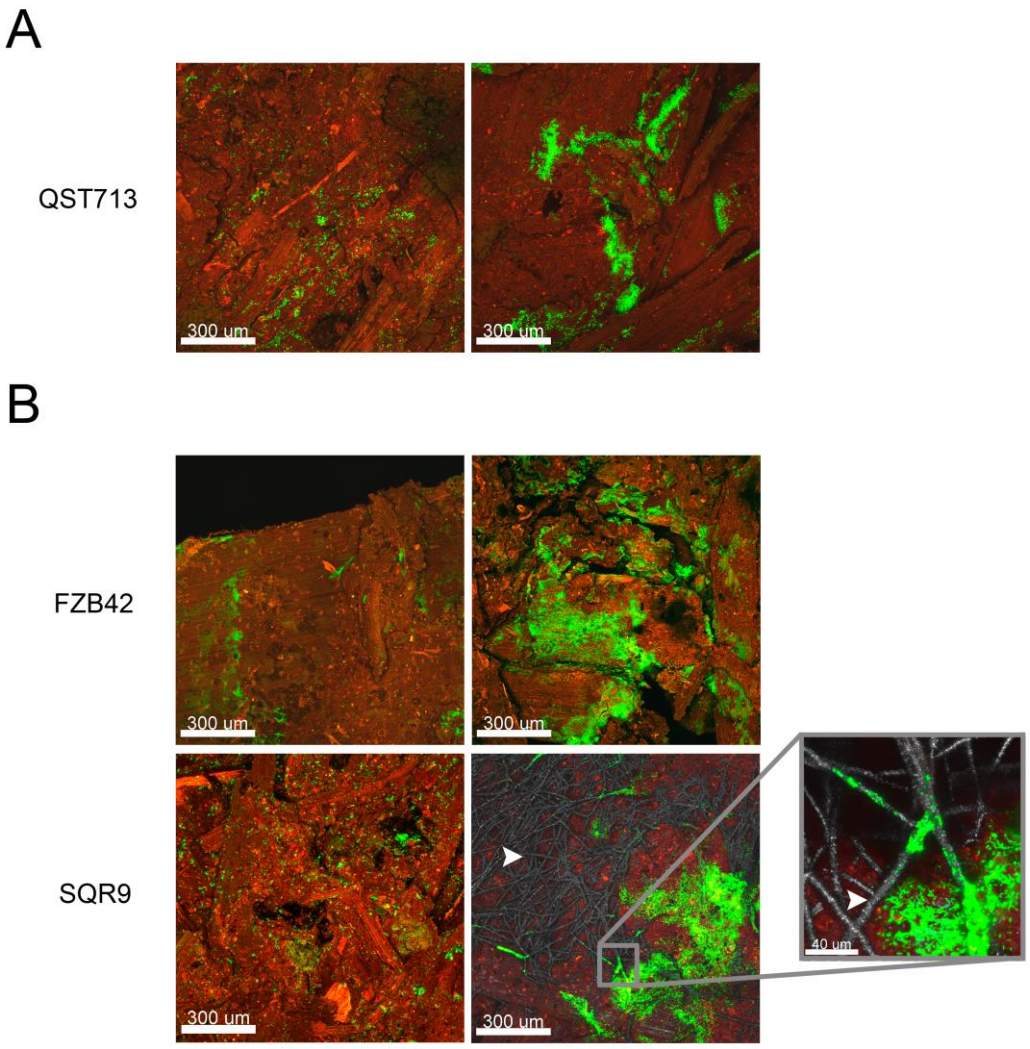

Fig. 5: *Bacillus* microcolonies and installed biofilm on the culture compost of *A. bisporus*. Confocal laser scanning microscopy of (A) *B. velezensis* QST713 (green) cultivated with *A. bisporus* and *T. aggressivum* in the compost (red). (B) *B. velezensis* FZB42 (green) or *B. velezensis* SQR9 (green) cultivated with *A. bisporus* (grey) and *T. aggressivum* in the compost. We observed a positive spatial interaction between the strain SQR9 and the mycelium of *A. bisporus* (white arrow) that colocalized.

To determine if other biocontrol strains of *B. velezensis* species form similar biofilm structures in compost co-cultured with *T. aggressivum* and *A. bisporus*, two other biocontrol *B. velezensis* isolates, FZB42-FB01 and SQR9-*gfp* were tested. Both strains showed, like strain QST713,

heterogeneous colonisation of the substrate with sparse biofilm structures (Fig. 5B). A specific spatial interaction of strain SQR9 (in green) and the mycelium of *A. bisporus* (contrasted in grey thanks to reflected light) was observed after 6 days of culture, suggesting a positive interaction between the two species such as a metabolic cooperation (Zhang et al., 2015).

Antagonism experiment in Petri dishes showed that the three *B. velezensis* strains were able to inhibit *T. aggressivum* similarly (Fig. S1). However, further investigations are needed to determine if the mechanisms of inhibition of *T. aggressivum* are similar with these three *B. velezensis* isolates.

4 Discussion

We previously reported an effective biocontrol effect of *B. velezensis* QST713 against *T. aggressivum* during the culture of *A. bisporus*. This protective effect is significant before seven days of culture, and the industrial compost is rich in strains assigned to *Bacillus velezensis* species (Pandin et al., 2018a, 2018b). We also observed that strain QST713 was capable of forming dense and robust biofilms on inert surfaces and to inhibit the growth of *T. aggressivum in vitro* (Pandin et al., 2018aIn this study, we attempted to explore the mechanisms of protection in a compost micromodel, including both the effective biofilm formation of strain QST713 in the compost and the involvement of antimicrobial compounds. The relative quantification of strain QST713 genes expression related to biofilm formation and antimicrobial production was assessed by qPCR, through the development of a simplified micromodel of *A. bisporus* culture with the addition of the pathogen *T. aggressivum*. An axenic industrial autoclaved compost was used to remove the native microbiota rich in *Bacillus* species and to focus specifically in the response of strain QST713 to the presence of *T. aggressivum* or/and *A. bisporus* in the compost.

The results showed that genes related to biofilm formation (*epsC, tap A* and *blsA*) were up-regulated and the motility gene *hag* strongly down-regulated in the compost compared to the exponential growth phase in broth compost extract. The swarming motility (*swrA*) was also active in the compost. The expression profile of strain QST713 in the compost was close to the stationary growth phase profile in the broth compost extract with the exception of the *hag* gene which was underexpressed and the *tapA* gene which was overexpressed, suggesting an effective biofilm formation in the compost. The large variability in gene expression in the compost samples could be related to the heterogeneity of the compost colonisation as shown by CLSM. In the same way, the expression profile of genes encoding secondary metabolites was close to the stationary growth phase profile in the broth compost extract with the exception of *fenA*, which was upregulated and *dhbA*, which was downregulated in the compost, suggesting a higher expression of *fenA* in the compost biofilm mode of life.

We also showed that *srfAA* and *fenA* were up-regulated in the presence of *T. aggressivum*, suggesting that surfactin and fengycin would be overproduced in the presence of the pathogen. It was also demonstrated in other *Bacillus* that fengycin synthesis could be regulated by the presence of pathogenss (Cawoy et al., 2015; Chowdhury et al., 2015) and that its toxicity varied, depending on the pathogen (Guo et al., 2014; Cawoy et al., 2015). Surfactin has been involved in biofilm formation (Aleti et al., 2016), swarming motility and environmental colonisation (Bais, 2004; Ghelardi et al., 2012), microbial communication as a signalling molecule that triggers matrix production *via* KinC, a sensor histidine kinase (López et al., 2009; López and Kolter, 2010), plant defences elicitor (Ongena and Jacques, 2008) and also as antifungal compound (Liu et al., 2014).

In order to evaluate the specificity of the behaviour of the strain QST713, we also studied two biocontrol strains for their ability to colonise the compost and to antagonize *T. aggressivum*. The strain FZB42 has recently been authorized as market biocontrol strain for mushroom cultivation in France (personal communication, french Technical Mushroom Centre (CTC)).

However, no public data are currently available neither on its effect on yield of *A. bisporus* nor on its biocontrol effect against *T. aggressivum.* The strain SQR9 is not currently used in France as a biocontrol agent. Both strains have genes related to biofilm formation, fengycin and surfactin production (Pandin et al., 2018a), and can inhibit the growth of *T. aggressivum in vitro* and in the compost. Spatial interaction of strain SQR9 and the mycelium of *A. bisporus* suggests a potential specific interaction between these organisms. Similar observations were reported with strain SQR9 used to protect maize crops, where the genes implicated in biofilm formation were up-regulated in the presence of root exudates (Zhang et al., 2015).

Further investigations are needed to formally assign the role of biofilm formation, surfactin and fengycin production in the biocontrol mechanisms that occur during the cultivation of *A. bisporus*. The use of biocontrol products formulated with microencapsulated biocontrol agents would permit their introduction in agrosystems in the biofilm form. The recent study of Ma et al. (2015) demonstrated a better persistence of the biocontrol agents at the entrance to the system, as well as a better biocontrol effect on pathogens.

Acknowledgments

C. Pandin is granted a doctoral fellowship by the Ile-de-France Region, DIM ASTREA (project n° ast150075). We thank the MIMA2 platform (www6.jouy.inra.fr/mima2) for accessing to the confocal microscope Leica SP8. We thank G. Condemine for providing the *Escherichia coli* GM48 strain. We thank J. M. Savoie for providing the *Trichoderma aggressivum* f. *europaeum* Ta2 strain Z. We thank R. Borriss and R. Zhang for providing the *Bacillus amyloliquefaciens* FZB42-FB01 and SQR9-*gfp* strains, respectively. We thank R. Védie and T. Rousseau for providing the *Agaricus bisporus* Amycel Delta strain and the industrial compost.

References

Aleti, G., Lehner, S., Bacher, M., Compant, S., Nikolic, B., Plesko, M., Schuhmacher, R., Sessitsch, A., Brader, G., 2016. Surfactin variants mediate species-specific biofilm formation and root colonization in *Bacillus*. *Environ. Microbiol.* 18, 2634–2645. https://doi.org/10.1111/1462-2920.13405

Bais, H.P., 2004. Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiol.* 134, 307–319. https://doi.org/10.1104/pp.103.028712

Branda, S.S., Chu, F., Kearns, D.B., Losick, R., Kolter, R., 2006. A major protein component of the *Bacillus subtilis* biofilm matrix. *Mol. Microbiol.* 59, 1229–1238. https://doi.org/10.1111/j.1365-2958.2005.05020.x

Cao, Y., Zhang, Z., Ling, N., Yuan, Y., Zheng, X., Shen, B., Shen, Q., 2011. *Bacillus subtilis* SQR 9 can control *Fusarium* wilt in cucumber by colonizing plant roots. *Biol. Fertil. Soils* 47, 495–506. https://doi.org/10.1007/s00374-011-0556-2

Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., Ongena, M., 2015. Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens. Microb. Biotechnol*. 8, 281–295. https://doi.org/10.1111/1751-7915.12238

Chowdhury, S.P., Uhl, J., Grosch, R., Alquéres, S., Pittroff, S., Dietel, K., Schmitt-Kopplin, P., Borriss, R., Hartmann, A., 2015. Cyclic lipopeptides of *Bacillus amyloliquefaciens* subsp. *plantarum* colonizing the lettuce rhizosphere enhance plant defense responses toward the bottom rot pathogen *Rhizoctonia solani. Mol. Plant-Microbe Interact.* 28, 984–995. https://doi.org/10.1094/MPMI-03-15-0066-R

Fan, B., Chen, X.H., Budiharjo, A., Bleiss, W., Vater, J., Borriss, R., 2011. Efficient colonization of plant roots by the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42, engineered to express green fluorescent protein. *J. Biotechnol.* 151, 303–311. https://doi.org/10.1016/j.jbiotec.2010.12.022

Ghelardi, E., Salvetti, S., Ceragioli, M., Gueye, S.A., Celandroni, F., Senesi, S., 2012. Contribution of surfactin and SwrA to flagellin expression, swimming, and surface motility in *Bacillus subtilis. Appl. Environ. Microbiol.* 78, 6540–6544. https://doi.org/10.1128/AEM.01341-12

Guo, Q., Dong, W., Li, S., Lu, X., Wang, P., Zhang, X., Wang, Y., Ma, P., 2014. Fengycin produced by *Bacillus subtilis* NCD-2 plays a major role in biocontrol of cotton seedling damping-off disease. *Microbiol. Res.* 169, 533–540. https://doi.org/10.1016/j.micres.2013.12.001

Jordon-Thaden, I.E., Chanderbali, A.S., Gitzendanner, M.A., Soltis, D.E., 2015. Modified CTAB and TRIzol Protocols Improve RNA Extraction from Chemically Complex Embryophyta. *Appl. Plant* Sci. 3, 1400105. https://doi.org/10.3732/apps.1400105

Kearns, D.B., Chu, F., Rudner, R., Losick, R., 2004. Genes governing swarming in *Bacillus subtilis* and evidence for

a phase variation mechanism controlling surface motility. *Mol. Microbiol.* 52, 357–369. https://doi.org/10.1111/j.1365-2958.2004.03996.x

Khezri, M., Ahmadzadeh, M., Jouzani, G.S., Behboudi, K., Ahangaran, A., Mousivand, M., Rahimian, H., 2011. Characterization of some biofilm-forming *Bacillus subtilis* strains and evaluation of their biocontrol potential against *Fusarium culmorum*. *J. Plant Pathol*. 93, 373–382. https://doi.org/10.4454/JPP.V93I2.1192

Kröber, M., Verwaaijen, B., Wibberg, D., Winkler, A., Pühler, A., Schlüter, A., 2016. Comparative transcriptome analysis of the biocontrol strain *Bacillus amyloliquefaciens* FZB42 as response to biofilm formation analyzed by RNA sequencing. *J. Biotechnol*. 231, 212–223. https://doi.org/10.1016/j.jbiotec.2016.06.013

Largeteau, M.L., Savoie, J.-M., 2010. Microbially induced diseases of *Agaricus bisporus*: biochemical mechanisms and impact on commercial mushroom production. *Appl. Microbiol. Biotechnol.* 86, 63–73. https://doi.org/10.1007/s00253-010-2445-2

Liu, J., Hagberg, I., Novitsky, L., Hadj-Moussa, H., Avis, T.J., 2014. Interaction of antimicrobial cyclic lipopeptides from *Bacillus subtilis* influences their effect on spore germination and membrane permeability in fungal plant pathogens. *Fungal Biol.* 118, 855–861. https://doi.org/10.1016/j.funbio.2014.07.004

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402–408. https://doi.org/10.1006/meth.2001.1262

López, D., Kolter, R., 2010. Extracellular signals that define distinct and coexisting cell fates in *Bacillus subtilis*. *FEMS Microbiol. Rev.* 34, 134–149. https://doi.org/10.1111/j.1574-6976.2009.00199.x

López, D., Vlamakis, H., Losick, R., Kolter, R., 2009. Paracrine signaling in a bacterium. *Genes Dev*. 23, 1631–1638. https://doi.org/10.1101/gad.1813709

Ma, X., Wang, X., Cheng, J., Nie, X., Yu, X., Zhao, Y., Wang, W., 2015. Microencapsulation of *Bacillus subtilis* B99-2 and its biocontrol efficiency against *Rhizoctonia solani* in tomato. *Biol. Control* 90, 34–41. https://doi.org/10.1016/j.biocontrol.2015.05.013

Mamoun, M.L., Savoie, J.M., Olivier, J.M., 2000. Interactions between the pathogen *Trichoderma harzianum* Th2 and *Agaricus bisporus* in mushroom compost. *Mycologia* 92, 233–240. https://doi.org/10.2307/3761556

Mielich-Süss, B., Lopez, D., 2015. Molecular mechanisms involved in *Bacillus subtilis* biofilm formation. *Environ. Microbiol.* https://doi.org/10.1111/1462-2920.12527

Ongena, M., Jacques, P., 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiol. h*ttps://doi.org/10.1016/j.tim.2007.12.009

Pandin, C., Le Coq, D., Canette, A., Aymerich, S., Briandet, R., 2017. Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? *Microb. Biotechnol.* 10, 719–734. https://doi.org/10.1111/1751-7915.12693

Pandin, C., Le Coq, D., Deschamps, J., Védie, R., Rousseau, T., Aymerich, S., Briandet, R., 2018a. Complete genome sequence of *Bacillus velezensis* QST713: A biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease. *J. Biotechnol*. 278, 10–19. https://doi.org/10.1016/j.jbiotec.2018.04.014

Pandin, C., Védie, R., Rousseau, T., Le Coq, D., Aymerich, S., Briandet, R., 2018b. Dynamics of compost microbiota during the cultivation of *Agaricus bisporus* in the presence of *Bacillus velezensis* QST713 as biocontrol agent against *Trichoderma aggressivum*. *Biol. Control* 127, 39–54. https://doi.org/10.1016/J.BIOCONTROL.2018.08.022

Romero, D., Aguilar, C., Losick, R., Kolter, R., 2010. Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc. Natl. Acad. Sci.* 107, 2230–2234. https://doi.org/10.1073/pnas.0910560107

van Gestel, J., Vlamakis, H., Kolter, R., 2015. From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. *PLoS Biol.* 13, e1002141. https://doi.org/10.1371/journal.pbio.1002141

Verhamme, D.T., Kiley, T.B., Stanley-Wall, N.R., 2007. DegU co-ordinates multicellular behaviour exhibited by *Bacillus subtilis. Mol. Microbiol.* 65, 554–568. https://doi.org/10.1111/j.1365-2958.2007.05810.x

Vlamakis, H., Aguilar, C., Losick, R., Kolter, R., 2008. Control of cell fate by the formation of an architecturally complex bacterial community. *Genes Dev*. 22, 945–953. https://doi.org/10.1101/gad.1645008

Vlamakis, H., Chai, Y., Beauregard, P., Losick, R., Kolter, R., 2013. Sticking together: Building a biofilm the *Bacillus subtilis* way. *Nat. Rev.* Microbiol. https://doi.org/10.1038/nrmicro2960

Wang, L., Stegemann, J.P., 2010. Extraction of high quality RNA from polysaccharide matrices using cetlytrimethylammonium bromide. *Biomaterials* 31, 1612–1618. https://doi.org/10.1016/j.biomaterials.2009.11.024

Xu, Z., Zhang, R., Wang, D., Qiu, M., Feng, H., Zhang, N., Shen, Q., 2014. Enhanced control of cucumber wilt disease by *Bacillus amyloliquefaciens* SQR9 by altering the regulation of its DegU phosphorylation. *Appl. Environ. Microbiol.* 80, 2941–2950. https://doi.org/10.1128/AEM.03943-13

Yi, Y., Kuipers, O.P., 2017. Development of an efficient electroporation method for rhizobacterial *Bacillus mycoides* strains. *J. Microbiol. Methods* 133, 82–86. https://doi.org/10.1016/j.mimet.2016.12.022

Zeriouh, H., de Vicente, A., Pérez-García, A., Romero, D., 2014. Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. *Environ. Microbiol.* 16, 2196–2211. https://doi.org/10.1111/1462-2920.12271

Zhang, G.Q., Bao, P., Zhang, Y., Deng, A.H., Chen, N., Wen, T.Y., 2011. Enhancing electro-transformation

competency of recalcitrant *Bacillus amyloliquefaciens* by combining cell-wall weakening and cell-membrane fluidity disturbing. *Anal. Biochem*. 409, 130–137. https://doi.org/10.1016/j.ab.2010.10.013

Zhang, N., Yang, D., Wang, D., Miao, Y., Shao, J., Zhou, X., Xu, Z., Li, Q., Feng, H., Li, S., Shen, Q., Zhang, R., 2015. Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates. *BMC Genomics* 16, 685. https://doi.org/10.1186/s12864-015-1825-5

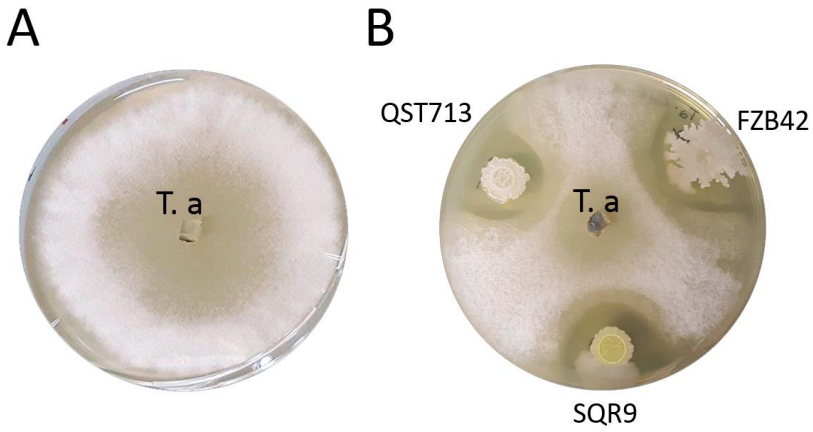

Fig. S1: Antagonism test of *T. aggressivum* by *B. velezensis* species: (A) *T. aggressivum* alone; (B) *T. aggressivum* with *B. velezensis* QST713, *B. velezensis* FZB42 and *B. velezensis* SQR9. The fungal pathogen was inoculated with an agar plug taken from a 72 h fungal culture that was deposited on the center of YMEA medium plate (yeast extract 2 g L^{-1}, malt extract 20 g L^{-1}, agar 15 g L^{-1}). 5 µL of sterile water (control) for (A) or of an overnight culture of *B. velezensis* in TSB for (B) was inoculated in three spots at 3 cm of *T. aggressivum* spot. Petri dishes were incubated at 25 °C and observations were performed at 4 days. Experiment was repeated 3 times.

DISCUSSION GENERALE

L'utilisation massive de produits phytosanitaires de synthèse dans les systèmes agricoles a mené par leur toxicité et leur persistance à un effondrement de la biodiversité sur la planète et à une pollution alarmante des écosystèmes. Aujourd'hui, la préservation de cette biodiversité et des écosystèmes naturels est une problématique mondiale récurrente face à laquelle le gouvernement Français a mis en place depuis 2008, un plan d'action visant à réduire progressivement l'utilisation de ces pesticides chimiques jusqu'à 50% à l'horizon 2025. Ce plan d'action, EcoPhyto, repose sur le remplacement progressif de ces pesticides de synthèse par des méthodes de lutte naturelles grâce à l'utilisation d'organismes vivants. L'une des méthodes les plus répandues dans le cas de maladies culturales d'origine microbiennes est l'utilisation de microorganismes naturels comme agents de biocontrôle, tels que les bactéries.

Aujourd'hui, l'utilisation d'agents de biocontrôle d'origine microbienne dans les cultures en champs apparait généralement efficace mais nécessite de renouveler les applications et reste soumise aux fluctuations climatiques. Une amélioration de leur performance s'impose donc, cela passe tout d'abord par une meilleure compréhension des mécanismes de bioprotection mis en œuvre par ces agents de biocontrôle au sein des écosystèmes. Les mécanismes d'action exercés par les agents de biocontrôle microbiens sont encore peu décrits à ce jour et reposent principalement sur l'antagonisme des bioagresseurs par la sécrétion de molécules antimicrobiennes, les phénomènes de compétition ou encore la stimulation des défenses naturelles des plantes. Au démarrage du projet en 2015, peu d'études s'intéressaient à la notion de biofilm comme partie intégrante de ces mécanismes de bioprotection, mettant de côté les capacités de colonisation, de résistance et de la physiologie spécifique des microorganismes associés à ces structures biologiques.

Au cours de ce travail de thèse, nous avons choisi d'étudier un modèle de culture non-soumis aux fluctuations climatiques et montrant des résultats d'efficacité reproductibles suite à l'utilisation d'un agent de biocontrôle bactérien. En effet, lors de la culture industrielle d'*Agaricus bisporus* en France, l'application unique du produit de biocontrôle Serenade ® en début de culture, permet avec succès le traitement du substrat de culture, contre *Trichoderma aggressivum*, un compétiteur majeur d'*A. bisporus* empêchant le développement mycélien du champignon de couche. Nous avons ainsi déterminé, (i) la capacité de la souche active de ce produit de biocontôle, *Bacillus subtilis* QST713, à former des biofilms et à inhiber le pathogène *T. aggressivum*, (ii) son impact sur les communautés microbiennes du compost de culture d'*A. bisporus* et leurs dynamiques, et (iii) par quels mécanismes l'effet de biocontrôle observé était mis en place.

La souche QST713, nouvellement renommée B. velezensis QST713 dispose de l'arsenal génétique nécessaire à la formation de biofilm et à la synthèse de différents antimicrobiens.

La souche QST713 étant inconnue des banques de données, nous avons entrepris de séquencer son génome et d'étudier ses spécificités génomiques reliées à la formation de biofilm et la production de métabolites secondaires. Comme nous l'avons vu précédemment (Synthèse bibliographique – Chapitre 1.3.2.1), la classification des espèces au sein du « *Bacillus subtilis* species complex » a subi une grande réorganisation ces dernières années (Fan et al., 2017). Lors de ce travail de thèse, l'utilisation d'outils et d'analyses bioinformatiques, tels que l'hybridation digitale ADN-ADN (dDDH : digital DNA-DNA hybridization) reproduisant informatiquement l'expérience lourde d'hybridation ADN-ADN, le calcul

de l'identité moyenne des séquences nucléotidiques (ANI : Average Nucleotide Identity) et l'identité moyenne des séquences protéiques (AAI : Average Amino acid Identity), nous ont permis d'obtenir de façon simple une classification taxonomique fiable réalisée sur 30 séquences génomiques de souches appartenant à ce complexe d'espèces. Grâce à ce travail, la souche QST713, appartenant initialement au groupe *B. subtilis*, a pu être reclassifiée parmi les *B. velezensis* du groupe opérationnel *B. amyloliquefaciens*. Avant la nouvelle classification, l'espèce *B. velezensis* était anciennement connue sous le nom de *B. amyloliquefaciens subsp. plantarum*, une espèce associée aux plantes (Dunlap et al., 2015, 2016).

A la lumière de cette nouvelle information, nous avons pris la décision de rechercher dans son génome la présence de gènes impliqués dans la formation de biofilm chez *B. subtilis*, une bactérie modèle du « *Bacillus subtilis* complex species », dont les caractéristiques et mécanismes moléculaires de la formation de biofilm ont été longuement étudiés (Vlamakis et al., 2013). Nous avons mis en évidence que la souche QST713 possédait les gènes impliqués dans la production de la matrice extracellulaire, composante structurelle majeure des biofilms, composée essentiellement d'exopolysaccharides synthétisés par les enzymes codées par l'opéron *epsA-O* (Branda et al., 2006), de fibres amyloïde TasA synthétisées par les enzymes codées par l'opéron *tapA-sipW-tasA* (De Jong et al., 2009; Romero et al., 2010), du polymère γ-glutamate donnant un aspect muqueux aux colonies et synthétisé par les enzymes codées par l'opéron *pgs* (Stanley and Lazazzera, 2005), et de l'hydrophobine BlsA, formant une couche hydrophobe à la surface du biofilm (Kobayashi and Iwano, 2012; Hobley et al., 2013, 2015; Kesel et al., 2016). La souche QST713 possède également les gènes permettant la détection des signaux extracellulaires et la régulation des gènes synthétisant la matrice extracellulaire. La synthèse de la matrice obéit à un réseau de régulation complexe impliquant une détection de signaux et la phosporylation du régulateur Spo0A *via* les senseurs histidines kinases membranaires (KinA, KinB, KinC et KinD). La molécule Spo0A phosphorylée (Spo0A~P) réprime l'expression de AbrB (répresseur de la synthèse de matrice) et la synthèse du répresseur de la synthèse de matrice, SinR, par le biais de l'activation de l'antirépresseur SinI (Chai et al., 2010; Vlamakis et al., 2013). La répression de SinR lève la répression de la synthèse de SlrR, ce qui permet l'expression de l'opéron *tasA* (Chu et al., 2008) et la repression du gène de motilité *hag* impliqués dans la synthèse de flagelline (Vlamakis et al., 2013), gène également présent chez la souche QST713. Spo0A~P intervient également dans la synthèse de surfactine (gènes *srf*), un lipopeptide tensio-actif impliqué dans la formation de biofilm, les systèmes de communication (López et al., 2009; Aleti et al., 2016) et la régulation des gènes du système de motilité swarming (gènes *swr*) (Kearns et al., 2004), gènes présents chez la souche QST713. *B. velezensis* QST713 possède également les gènes régulateurs *degU* et *comA*. DegU est impliqué dans la régulation de la synthèse de matrice, de la motilité swarming (Kearns et al., 2004; Verhamme et al., 2007; Vlamakis et al., 2013; Xu et al., 2014; Mielich-Süss and Lopez, 2015). ComA, quant à lui, contrôle la production de surfactine et les gènes de compétence *com* permettant d'incorporer l'ADN exogène.

Nous avons également vu précédemment (Synthèse bibliographique – Chapitre 1.3.2.1) que la synthèse de métabolites secondaires, comprenant en particulier les antimicrobiens, faisait partie intégrante des mécanismes de bioprotection exercés par les agents de biocontrôle. Nous avons donc recherché, *via* le webserveur antiSMASH dédié à la recherche de cluster de métabolites secondaires au sein de séquences génomiques, la présence de tels clusters dans le génome de la souche QST713. Les résultats obtenus ont révélé que cette souche possédait quinze clusters de gènes impliqués dans la synthèse de métabolites secondaires, dont neuf connus impliqués dans la production d'antimicrobiens (surfactine, bacillomycine D, fengycine, macrolactine, difficidine, bacillaene, bacilysine, éricine) et

d'un sidérophore (bacillibactine). Deux clusters codant potentiellement de nouveaux antimicrobiens de fonctions inconnues à ce jour ont été trouvés dans le génome de *B. velezensis* QST713. Le premier arbore les gènes de synthèse de peptides non-ribosomiques (NRPS) et le second des gènes de synthèse de policétides et de peptides non-ribosomiques (TransATPKS-NRPS). Une étude de Cai et al. (2017) a comparé la présence de ces deux clusters chez 37 souches appartenant au *Bacillus subtilis* species complex ne prenant pas en compte la souche QST713. Douze souches appartenant à l'espèce *B. velezensis*, possédaient l'un ou l'autre cluster. Seule la souche CC09 dans cette étude possédait les deux. Les auteurs de cette étude se sont également intéressés à l'enchaînement d'acides aminés prédit par antiSMASH pour ces deux clusters chez ces douze souches. Dans le cas du cluster NRPS, deux types de structures ont été trouvées pour dix des souches. Dans notre étude, la souche QST713 possédait une structure de ce NRPS encore différente de ces deux types, composée de l'enchainement d'acides aminés Leucine-Cystéine-Sérine-Cystéine-Alanine-Phénylalanine-Asparagine-Asparagine. Pour quatre souches, le cluster TransATPKS-NRPS comportait 3 structures différentes, celui chez la souche QST713 arborant la même structure que celui de la souche CC09 (Cai et al., 2017). Ces résultats suggèrent l'appartenance de ces potentiels métabolites à l'espèce *B. velezensis*, la variabilité dans la structure de ces métabolites pourrait correspondre à des cibles potentiellement différentes ou à une variation dans la toxicité de ces composés comme cela est le cas pour la fengycine (Guo et al., 2014; Cawoy et al., 2015).

Ces premieres observations *in silico* ont été confortées phénotypiquement par des expériences de formations de biofilms (immergés, pellicules et macrocolonies), de motilité (swarming) et d'inhibition de *T. aggressivum in vitro*. *B. velezensis* QST713 était capable de former des biofilms robustes ayant une structure tridimensionnelle complexe riche en matrice extracellulaire avec un aspect muqueux. La capacité de colonisation d'un milieu du type swarming de la souche QST713 s'est révélée similaire à celle d'une autre souche de biocontrôle, *B. velezensis* SQR9 mais en dessous de la performance de la souche *B. velezensis* FZB42. La comparaison de la capacité à former des biofilms de la souche QST713 avec *B. velezensis* SQR9 et *B. velezensis* FZB42, a montré que la souche QST713 est un agent de biocontrôle efficace en terme de formation de biofilm spatiallement organisé. La capacité de la souche QST713 à inhiber *T. aggressivum* a montré d'une part, une forte inhibition de cette moisissure dans la zone entourant la bactérie, et d'autre part, une inhibition de la sporulation de la moisissure possiblement par l'émission de composés organiques volatiles limitant de ce fait sa dissémination (Raza et al., 2016; Sharifi and Ryu, 2016; Besset-Manzoni et al., 2018).

Bacillus velezensis QST713 part en guerre contre Trichoderma aggressivum !

La protection des cultures par d'autres systèmes que les pesticides chimiques est à l'ordre du jour, partout dans le monde. De plus en plus de recherche sont effectuées sur l'impact qu'ont ces agents de biocontrôle utilisés pour protéger les cultures des bioagresseurs, sur les communautés microbiennes au sein des cultures (Andreote et al., 2009; Edel-Hermann et al., 2009; Kim et al., 2010). Au démarrage du projet, peu d'informations étaient disponibles concernant la diversité microbienne du compost utilisé dans les cultures d'*A. bisporus* en France et une forte demande de la filière nous a conduit à réaliser cette étude. Une large partie de ce travail de thèse a consisté à (i) déterminer la composition microbienne du compost industriel grâce au séquençage de la région V4-V5 de l'ADNr 16S pour l'identification bactérienne et au séquençage de l'ITS2 (internal transcribed spacer) pour l'identification des populations

fongiques, (ii) étudier l'impact de *B. velezensis* QST713 sur les communautés microbiennes du compost et sur le compétiteur *T. aggressivum* lors de la culture d'*A. bisporus*.

Le compost naturel était dominé par les taxons des phylums Firmicutes, Protéobactéries, Bacteroidetes et Actinobactéries pour les communautés bactériennes, et par les taxons des phylums Ascomycètes principalement pour les champignons. Les résultats ont révélé que la souche QST713 avait un impact transitoire sur la structure des communautés microbiennes du compost. A noter qu'un effet similaire était observé en présence de *T. aggressivum* ajouté seul dans le compost. Ces résultats suggèrent, non pas un effet spécifique, mais plutôt une réponse des communautés à l'introduction d'un nouvel organisme dans le système. Cet « effet intrus », a montré un changement dans la structure des communautés traduisant un déséquilibre des phénomènes de compétition pour la même niche écologique déjà existants au sein du compost. Ce déséquilibre était le plus significatif à quinze jours, mais ne perdurait pas lors de la culture montrant une résilience efficace du système. De la même façon que lors des expériences d'inhibition *in vitro*, nous avons pu observer une inhibition efficace de *T. aggressivum* lors de la présence de *B. velezensis* QST713 dans le compost. Cette inhibition débutait en début de phase végétative, avant le septième jour de culture. Nous avons également observé une augmentation des *Bacillaceae*, sans effet de biocontrôle en présence de *T. aggressivum* seul, ce qui suggère que l'effet de biocontrôle observé sur *T. aggressivum* en présence de la souche QST713 serait spécifiquement dû à l'introduction de cette souche. Le compost est naturellement riche en microorganismes dégradeurs et en potentiels agents de biocontrôle, et peut être révalorisé après la culture d'*A. biporus* pour fertiliser les sols dans d'autres cultures en plein champs (Ntougias et al., 2004; Noble and Coventry, 2005; Mehta et al., 2013, 2014).

La composition des communautés microbiennes obtenue grâce au séquençage de l'ADN 16s et ITS2 ne tient pas compte de l'état physiologique des populations présentes. De récentes études ont apporté une information sur les populations actives du compost en réalisant à la fois le séquençage de la région V4 de l'ADNr 16S et des ITS, et des ADNc issus de la transcription réverse des ARN totaux présents dans le compost au cours de la culture d'*A. bisporus* (McGee et al., 2017a, 2017b; McGee, 2018). Ces approches ont montré en particulier que l'analyse des ADNc comparée à celle des ADN générait un nombre d'OTUs (unité taxonomique opérationnelle) plus faible, suggérant que certaines OTUs présentes dans l'analyse ADN étaient soit dormantes soit mortes (McGee et al., 2017b). Par ailleurs, dans ces études, les populations d'abondances plus élevées n'étaient pas systématiquement les plus actives. Les Protéobactéries, par exemple, étaient très abondantes au lardage (jour 0), mais n'étaient que peu actives à ce stade du procédé. Au contraire les Firmicutes étaient plus actifs qu'abondants au même stade (McGee et al., 2017a).

Les mécanismes impliqués dans l'antagonisme de *T. aggressivum* par *B. velezensis* QST713 ne sont pas encore formellement décrits aujourd'hui. Ces mécanismes antagonistes pourraient être une combinaison (i) d'exclusion de niche de *T. aggressivum* par des biofilms compétitifs, (ii) de sécrétion de molécules antifongiques et (iii) de sécrétion de molécules de signalisation (composés organiques volatiles, molécules signal de quorum-sensing ou antimicrobiens) permettant d'induire une réponse de défense d'*A. bisporus* et de la flore autochtone du compost. Il serait intéressant de réaliser une étude du metatranscriptome dans le compost afin de définir les groupes de gènes fonctionnels les plus actifs lors de l'effet de biocontrôle de *B. velezensis* QST713 contre *T. aggressivum*.

Résister à l'envahissement de T. aggressivum, peut-être pas seule, mais ensemble !

Au regard des premiers résultats obtenus lors de l'analyse de la diversité bactérienne du compost et de l'effet de biocontrôle de *B. velezensis* QST713, nous avons tout d'abord pensé à un effet spécifique de la souche QST713 sur *T. aggressivum*. Pour démontrer cet effet, nous avons tenté de construire des mutants par délétion de gènes impliqués dans la formation de biofilm et la synthèse de métabolites secondaires afin de tester ces mutants dans le système de culture d'*A. bisporus*. Nous avons été confrontés à la difficulté à transformer la souche QST713 par des plasmides. De nombreux essais par des techniques de transformation naturelle (Jarmer et al., 2002), d'électroporation (Vehmaanperä, 1989; Kunst and Rapoport, 1995; Koumoutsi et al., 2004; Idris et al., 2007; Roh et al., 2009; Cao et al., 2011), de conjugaison (Fortineau et al., 2000; Trieu-Cuot et al., 1991), de tribotransformation (Yoshida and Sato, 2009) sont restés infructueux. Deux études, décrivant une méthode de transformation par électroporation de *Bacillus* récalcitrants (Zhang et al., 2011; Yi and Kuipers, 2017), nous ont permis d'obtenir avec difficulté des transformants de la souche QST713, dans laquelle nous avons introduit un plasmide portant les gènes nécessaires à la synthèse d'une protéine fluorescente, la Gfp (green fluorescent protein). Cette nouvelle souche nous a permis de visualiser spécifiquement la souche QST713-*gfp* fluorescente en microscopie confocale à balayage laser dans le compost. Cette technique de transformation est basée sur l'utilisation de plasmides non-méthylés, de glycine permettant l'affaiblissement de l'épais peptidoglycane des *Bacillus*, et d'un milieu de culture à pression osmotique élevée. Cependant, la transformation de la souche QST713 reste extrêmement compliquée aujourd'hui, et cette méthode n'a pas permis d'obtenir de résultats reproductibles et une efficacité de transformation suffisante pour permettre la construction d'une série de mutants ciblés. Nous avons donc choisi de réaliser une étude de l'expression transcriptionnelle de gènes impliqués dans la formation de biofilm et synthèse de métabolites secondaires chez *B. velezensis* QST713, en interaction avec *T. aggressivum* et/ou *A. bisporus* dans un compost autoclavé, permettant d'éliminer la flore naturelle du compost en bactéries du genre *Bacillus* potentiellement interférentes.

Grâce au micromodèle de culture simplifiée à trois partenaires en compost axénique que nous avons développé au laboratoire, nous avons pu déterminer l'état physiologique de *B. velezensis* QST713 dans le compost lors de la culture d'*A. bisporus.* La surexpression des gènes impliqués dans la formation de biofilm (*epsC, tapA*), ainsi que la sous-expression du gène de motilité *hag* chez la souche QST713 cultivée seule dans le compost en comparaison à la phase de croissance exponentielle en extrait de compost liquide (planctonique), nous a permis de statuer que la souche QST713 était majoritairement sous forme de biofilm dans cet environnement. Ces résultats ont été confortés par les analyses de microscopie confocale non-invasives dans les micromodèles à l'aide de la souche QST713-*gfp*. Lors de notre expérience sur l'expression transcriptionnelles de gènes chez *B. velezensis* QST713, les gènes impliqués dans la synthèse de fengycine et surfactine se sont avérés surexprimées en présence de *T. aggressivum*. Ces deux molécules sont produites par de nombreux *Bacillus* (*amyloliquefaciens*, *velezensis*, etc…). Ceci pourrait suggérer que l'effet de biocontrôle observé dans ce système de culture complexe, à l'instar d'être opéré spécifiquement par la souche QST713, s'effectuerait également par des réponses de la flore naturelle en réponse au protéome de la souche QST713. En effet, dans le système de culture complexe, de nombreuses bactéries des familles *Bacillaceae et Paenibacillaceae* sont présentes et d'abondances relatives plus élevées en présence de *B. velezensis* QST713 et de *B. velezensis* QST713/*T. aggressivum* (Fig. 12). L'apport de la souche QST713 permettrait donc d'une part, (i) une concentration de fengycine supérieure à celle présente dans le compost naturel permettant une meilleure inhibition de *T. aggressivum*, et d'autres part, (ii) une communication microbienne à plus grande échelle

grâce à la production de surfactine, en tant que molécule signal (activation du quorum-sensing, formation de biofilm). Ces signaux de communications, captés par la flore autochtone du compost, permettrait à cette flore de réagir, par exemple, en formant des barrières protectrices impliquant la formation de biofilm et la surexpression de métabolites secondaires (Abd El Daim et al., 2015; Timmusk et al., 2005). La surfactine, comme la fengycine, sont connues pour avoir une activité fongicide (Guo et al., 2014; Jia et al., 2015). Cependant, nous n'avons pas déterminé, au cours de cette étude, l'effet spécifique de l'une ou l'autre molécule sur *T. aggressivum* et ne pouvons formellement démontrer leur implication dans son inhibition.

Il serait intéressant d'étudier le transcriptome complet de la souche QST713 dans ces micromodèles simplifiés par RNA-sequencing, ce qui permettrait d'évaluer de façon plus générale sa réponse en présence d'*A. bisporus* ou *T. aggressivum*. De même, nous pourrions étudier l'impact des exsudats d'*A. bisporus*, *T. aggressivum* ou extrait de compost naturel sur la réponse de la souche QST713 comme cela a été réalisé dans les systèmes « plantes » (Zhang et al., 2015). De la même façon, l'ajout d'une seconde souche du genre *Bacillus* et l'étude de son transcriptome en présence de la souche QST713 dans ce pathosystème permettrait d'explorer l'hypothèse d'une réponse coordonnée de différentes populations microbiennes. L'utilisation de ce micromodèle de culture, combiné à des techniques de manipulation génétique des souches de biocontrôle et d'imagerie chimique non-invasive, telle que la spectroscopie infrarouge à transformée de Fourier (IRTF) ou l'imagerie par spectrométrie de masse, permettraient d'analyser *in situ* la diversité et la répartition spatiale des métabolites produits dans ce système. En conséquence, il serait possible d'identifier les substances ayant une interaction avec les différents partenaires du système étudié. De même, l'utilisation de méthode telle que l'HPLC (High-Performance Liquid Chromatography) réalisée sur des extraits de gélose se trouvant dans la zone d'inhibition lors des tests d'inhibition du pathogène par les agents de biocontrôle sur boite de Pétri, permettrait l'identification et la quantification des molécules intervenant dans cette inhibition (Xu et al., 2014).

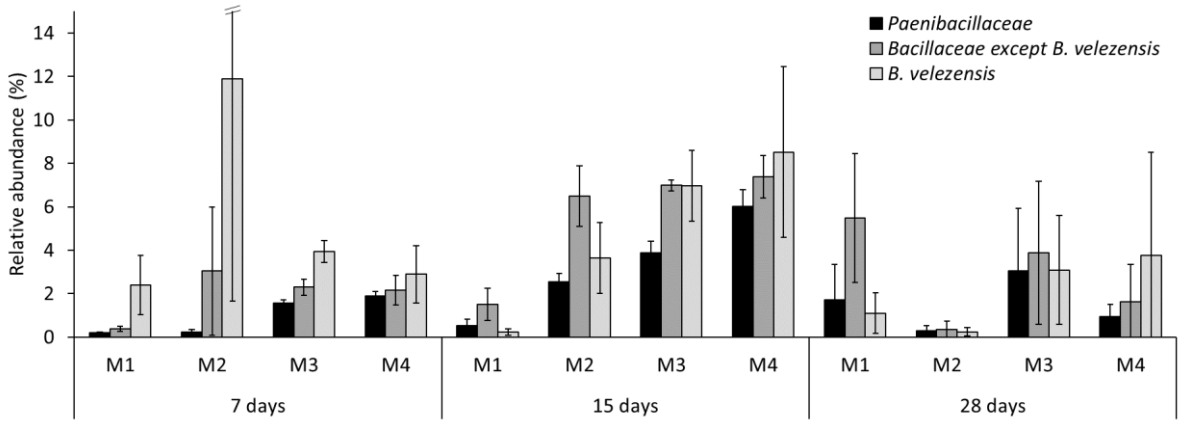

Figure 12: Abondance relative des *Paenibacillaceae*, *Bacillaceae* (excepté *B. velezensis*) et *B. velezensis* en présence ou non de *B. velezensis* QST713 et *T. aggressivum* lors des 28 premiers jours de culture d'*A. bisporus*. *A. bisporus* a été cultivé en présence (QST713 +) ou absence (QST713 -) de traitement par *B. velezensis* QST713 et avec (T. a +) ou sans (T. a -) inoculation avec *T. aggressivum*. Quatre conditions ont été réalisées: (M1) T. a -, QST713 -; (M2) T. a +, QST713 -; (M3) T. a -, QST713 +; (M4) T. a +, QST713 +. Les barres représentent les écarts-types des moyennes des échantillons (n = 3).

Un long chemin reste encore à parcourir dans la compréhension de ces mécanismes d'interactions entre espèces dans cet écosystème complexe. Quels sont les signaux reconnus par *B. velezensis* QST713 en présence de *T. aggressivum* et *A. bisporus*, mais également en présence de la flore autochtone du compost ? Quelles voies de régulations les réponses à ces signaux empruntent-t-elles ? Quels systèmes de communication sont utilisés par *B. velezensis* QST713 dans le compost ? Quelles réponses de la flore naturelle du compost observe-t-on ? Quelles réponses d'*A. bisporus* et *T. aggressivum* observe-t-on face à l'introduction de *B. velezensis* QST713 dans ce pathosystème ?

Amélioration des systèmes de biocontrôle.

Le système de culture de champignons de couche, bien qu'étant un système fermé, n'empêche en aucun cas l'apparition de phénomènes de résistances du pathogène à l'agent de biocontrôle utilisé (Savoie et al., 2001). Nous avons été confrontés lors de ce projet, à ce que nous pensions être des contaminations par d'autres *T. aggressivum.* Il se peut que ces contaminations aient été en réalité l'apparition d'un variant de cette souche ayant développé une résistance contre la souche QST713. Ce phénomène a déjà été observé chez *T. aggressivum* et *Botrytis cinerea* (Szczech et al., 2008; Jiang et al., 2009). Pour pallier à ce potentiel problème émergent pour la filière, une possible rotation de différentes souches de biocontrôle ou des cocktails de souches pourraient limiter l'emergence de variants résistants. Récemment, l'agent de biocontrôle *B. velezensis* FZB42 a reçu une autorisation de mise sur le marché comme agent de biocontrôle dans la filière des champignons de couche. Actuellement, aucune donnée n'est disponible sur l'efficacité de cet agent dans ce système. Cependant, la souche FZB42 est génétiquement manipulable et permettrait une étude mécanistique formelle des mécanismes de biocontrôle par criblage de mutants et de leurs effets dans ce système. De même, une rotation de variétés d'*A. bisporus* permettrait d'enrayer la sélection de souches pathogènes adaptées aux métabolites produits par le champignon.

Un autre phénomène se produit lors de l'utilisation de souches de biocontrôle dans les systèmes agricoles. Les agents de biocontrôle du type *Bacillus*, sont généralement formulés sous forme de poudre mouillable à pulvériser et leur survie dans les écosystèmes n'est pas optimale, engendrant une perte d'efficacité. De récentes études sur la formulation de produits à base de *Bacillus* ont montré qu'une encapsulation de ces bactéries dans des billes composées de maltodextrine et de gomme arabique permettait une formation préalable de biofilm, une meilleure survie des bactéries et un meilleur effet de biocontrôle contre *Rhizoctonia solani* dans les cultures de tomates (Ma et al., 2015). L'utilisation d'un support nutritif naturel, mélangé à la poudre mouillable contenant l'agent de biocontrôle avant son introduction dans le système de culture, permettrait d'une part, l'initiation de la formation de biofilm par l'agent et une meilleure survie au moment de l'entrée dans le système de culture.

REFERENCES

Abd El Daim, I.A., Häggblom, P., Karlsson, M., Stenström, E., Timmusk, S., 2015. *Paenibacillus polymyxa* A26 Sfp-type PPTase inactivation limits bacterial antagonism against *Fusarium graminearum* but not of *F. culmorum* in kernel assay. *Front. Plant Sci*. 06, 368. https://doi.org/10.3389/fpls.2015.00368

Abou-Zeid, M.A., 2012. Pathogenic variation in isolates of *Pseudomonas* causing the brown blotch of cultivated mushroom, *Agaricus bisporus*. *Brazilian J. Microbiol.* 43, 1137–1146. https://doi.org/10.1590/S1517-83822012000300041

Abubaker, K.S., Sjaarda, C., Castle, A.J., 2013. Regulation of three genes encoding cell-wall-degrading enzymes of *Trichoderma aggressivum* during interaction with *Agaricus bisporus*. Can. *J. Microbiol*. 59, 417–424. https://doi.org/10.1139/cjm-2013-0173

Aleti, G., Lehner, S., Bacher, M., Compant, S., Nikolic, B., Plesko, M., Schuhmacher, R., Sessitsch, A., Brader, G., 2016. Surfactin variants mediate species-specific biofilm formation and root colonization in *Bacillus*. *Environ. Microbiol*. 18, 2634–2645. https://doi.org/10.1111/1462-2920.13405

Aleti, G., Sessitsch, A., Brader, G., 2015. Genome mining: Prediction of lipopeptides and polyketides from *Bacillus* and related Firmicutes. *Comput. Struct. Biotechnol. J.* https://doi.org/10.1016/j.csbj.2015.03.003

Andreote, F.D., De Araújo, W.L., De Azevedo, J.L., Van Elsas, J.D., Da Rocha, U.N., Van Overbeek, L.S., 2009. Endophytic colonization of potato (Solanum tuberosum L.) by a novel competent bacterial endophyte, *Pseudomonas putida* strain P9, and its effect on associated bacterial communities. *Appl. Environ. Microbiol*. 75, 3396–3406. https://doi.org/10.1128/AEM.00491-09

ANICC, 2014. Les fruits et légumes transformés en France.

Barrios, E., 2007. Soil biota, ecosystem services and land productivity. *Ecol. Econ*. 64, 269–285. https://doi.org/10.1016/j.ecolecon.2007.03.004

Berendsen, R.L., Kalkhove, S.I.C., Lugones, L.G., Wösten, H.A.B., Bakker, P.A.H.M., 2012. Germination of *Lecanicillium fungicola* in the mycosphere of *Agaricus bisporus*. *Environ. Microbiol. Rep*. 4, 227–233. https://doi.org/10.1111/j.1758-2229.2011.00325.x

Besset-Manzoni, Y., Rieusset, L., Joly, P., Comte, G., Prigent-Combaret, C., 2018. Exploiting rhizosphere microbial cooperation for developing sustainable agriculture strategies. *Environ. Sci. Pollut. Res.* 1–18. https://doi.org/10.1007/s11356-017-1152-2

Bonmatin, J.-M., Laprevote, O., Peypoux, F., 2003. Diversity Among Microbial Cyclic Lipopeptides: Iturins and Surfactins. Activity-Structure Relationships to Design New Bioactive Agents. *Comb. Chem. High Throughput Screen*. 6, 541–556. https://doi.org/10.2174/138620703106298716

Borriss, R., 2015. Bacillus, a plant-beneficial bacterium, *in:* Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture. *Springer International Publishing, Cham*, pp. 379–391. https://doi.org/10.1007/978-3-319-08575-3_40

Branda, S.S., Chu, F., Kearns, D.B., Losick, R., Kolter, R., 2006. A major protein component of the *Bacillus subtilis* biofilm matrix. *Mol. Microbiol.* 59, 1229–1238. https://doi.org/10.1111/j.1365-2958.2005.05020.x

Branda, S.S., González-Pastor, J.E., Dervyn, E., Ehrlich, S.D., Losick, R., Kolter, R., 2004. Genes involved in formation of structured multicellular communities by *Bacillus subtilis*. *J. Bacteriol.* 186, 3970–3979. https://doi.org/10.1128/JB.186.12.3970-3979.2004

Breukink, E., Wiedemann, I., Van Kraaij, C., Kuipers, O.P., Sahl, H.G., De Kruijff, B., 1999. Use of the cell wail precursor lipid II by a pore-forming peptide antibiotic. *Science* (80-.). 286, 2361–2364. https://doi.org/10.1126/science.286.5448.2361

Cai, X.C., Liu, C.H., Wang, B.T., Xue, Y.R., 2017. Genomic and metabolic traits endow *Bacillus velezensis* CC09 with a potential biocontrol agent in control of wheat powdery mildew disease. *Microbiol. Res*. 196, 89–94. https://doi.org/10.1016/j.micres.2016.12.007

Cao, Y., Zhang, Z., Ling, N., Yuan, Y., Zheng, X., Shen, B., Shen, Q., 2011. *Bacillus subtilis* SQR 9 can control *Fusarium* wilt in cucumber by colonizing plant roots. *Biol. Fertil. Soils* 47, 495–506. https://doi.org/10.1007/s00374-011-0556-2

Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., Ongena, M., 2015a. Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. *Microb.*

Biotechnol. 8, 281–295. https://doi.org/10.1111/1751-7915.12238

Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., Ongena, M., 2015b. Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. *Microb. Biotechnol*. 8, 281–295. https://doi.org/10.1111/1751-7915.12238

Chai, Y., Kolter, R., Losick, R., 2010. Reversal of an epigenetic switch governing cell chaining in *Bacillus subtilis* by protein instability. *Mol. Microbiol.* 78, 218–229. https://doi.org/10.1111/j.1365-2958.2010.07335.x

Chen, X.-H., Vater, J., Piel, J., Franke, P., Scholz, R., Schneider, K., Koumoutsi, A., Hitzeroth, G., Grammel, N., Strittmatter, A.W., Gottschalk, G., Sussmuth, R.D., Borriss, R., 2006. Structural and Functional Characterization of Three Polyketide Synthase Gene Clusters in *Bacillus amyloliquefaciens* FZB 42. *J. Bacteriol.* 188, 4024–4036. https://doi.org/10.1128/JB.00052-06

Chen, X.H., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Süssmuth, R., Piel, J., Borriss, R., 2009a. Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. *J. Biotechnol*. 140, 27–37. https://doi.org/10.1016/j.jbiotec.2008.10.011

Chen, X.H., Scholz, R., Borriss, M., Junge, H., Mögel, G., Kunz, S., Borriss, R., 2009b. Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. *J. Biotechnol.* 140, 38–44. https://doi.org/10.1016/j.jbiotec.2008.10.015

Chowdhury, S.P., Hartmann, A., Gao, X.W., Borriss, R., 2015a. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42 - A review. *Front. Microbiol.* 6, 780. https://doi.org/10.3389/fmicb.2015.00780

Chowdhury, S.P., Uhl, J., Grosch, R., Alquéres, S., Pittroff, S., Dietel, K., Schmitt-Kopplin, P., Borriss, R., Hartmann, A., 2015b. Cyclic Lipopeptides of *Bacillus amyloliquefaciens* subsp. *plantarum* Colonizing the Lettuce Rhizosphere Enhance Plant Defense Responses Toward the Bottom Rot Pathogen Rhizoctonia solani. *Mol. Plant-Microbe Interact.* 28, 984–995. https://doi.org/10.1094/MPMI-03-15-0066-R

Chu, F., Kearns, D.B., McLoon, A., Chai, Y., Kolter, R., Losick, R., 2008. A novel regulatory protein governing biofilm formation in *Bacillus subtilis. Mol. Microbiol.* 68, 1117–1127. https://doi.org/10.1111/j.1365-2958.2008.06201.x

Colauto, N.B., Fermor, T.R., Eira, A.F., Linde, G.A., 2016. *Pseudomonas putida* stimulates primordia on *Agaricus bitorquis*. *Curr. Microbiol.* 72, 482–488. https://doi.org/10.1007/s00284-015-0982-8

Costerton, J.W., Geesey, G.G., Cheng, K.J., 1978. How bacteria stick. *Sci. Am.* 238, 86–95. https://doi.org/10.1038/scientificamerican0178-86

Costerton, J.W., Irvin, R.T., Cheng, K.J., Sutherland, I.W., 1981. The role of bacterial surface structures in pathogenesis. *Crit. Rev. Microbiol.* 8, 303–338. https://doi.org/10.3109/10408418109085082

Costerton, J.W., Lewandowski, Z., DeBeer, D., Caldwell, D., Korber, D., James, G., 1994. Biofilms, the customized microniche. *J. Bacteriol.* https://doi.org/10.1128/jb.176.8.2137-2142.1994

De Jong, W., Wösten, H.A.B., Dijkhuizen, L., Claessen, D., 2009. Attachment of *Streptomyces coelicolor* is mediated by amyloida fimbriae that are anchored to the cell surface via cellulose. *Mol. Microbiol.* 73, 1128–1140. https://doi.org/10.1111/j.1365-2958.2009.06838.x

Di Poi, C., Costil, K., Bouchart, V., Halm-Lemeille, M.-P., 2017. Toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. *Environ. Sci. Pollut. Res.* 25, 6122–6134. https://doi.org/10.1007/s11356-017-9306-9

Dong, Y.H., Wang, L.H., Xu, J.L., Zhang, H.B., Zhang, X.F., Zhang, L.H., 2001. Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. *Nature* 411, 813–817. https://doi.org/10.1038/35081101

Dunlap, C.A., Kim, S.J., Kwon, S.W., Rooney, A.P., 2016. *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens*; *Bacillus methylotrophicus*, *Bacillus amyloliquefaciens* subsp. *plantarum* and *'Bacillus oryzicola*' are later heterotypic synonyms of *Bacillus velezensis* based on phylogenom. *Int. J. Syst. Evol. Microbiol.* 66, 1212–1217. https://doi.org/10.1099/ijsem.0.000858

Dunlap, C.A., Kim, S.J., Kwon, S.W., Rooney, A.P., 2015. Phylogenomic analysis shows that *Bacillus amyloliquefaciens* subsp. *plantarum* is a later heterotypic synonym of *Bacillus methylotrophicus*. *Int. J. Syst. Evol. Microbiol.* 65, 2104–2109. https://doi.org/10.1099/ijs.0.000226

Edel-Hermann, V., Brenot, S., Gautheron, N., Aimé, S., Alabouvette, C., Steinberg, C., 2009. Ecological fitness of the biocontrol agent *Fusarium oxysporum* Fo47 in soil and its impact on the soil microbial communities. *FEMS Microbiol. Ecol.* 68, 37–45. https://doi.org/10.1111/j.1574-6941.2009.00656.x

EPA Office of Pesticide Programs, U., 2000. Technical Document for *Bacillus subtilis* QST713 also referred to as a BRAD. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006479_9-Aug-06.pdf

Fan, B., Blom, J., Klenk, H.P., Borriss, R., 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "operational group *B. amyloliquefaciens*" within the *B. subtilis* species complex. *Front. Microbiol*. 8, 22. https://doi.org/10.3389/fmicb.2017.00022

Federhen, S., Rossello-Mora, R., Klenk, H.P., Tindall, B.J., Konstantinidis, K.T., Whitman, W.B., Brown, D., Labeda, D., Ussery, D., Garrity, G.M., Colwell, R.R., Hasan, N., Graf, J., Parte, A., Yarza, P., Goldberg, B., Sichtig, H., Karsch-Mizrachi, I., Clark, K., McVeigh, R., Pruitt, K.D., Tatusova, T., Falk, R., Turner, S., Madden, T., Kitts, P., Kimchi, A., Klimke, W., Agarwala, R., DiCuccio, M., Ostell, J., 2016. Meeting report: GenBank microbial genomic taxonomy workshop (12-13 May, 2015). *Stand. Genomic Sci.* 11, 15. https://doi.org/10.1186/s40793-016-0134-1

Flemming, H.C., Neu, T.R., Wozniak, D.J., 2007. The EPS matrix: The "House of Biofilm Cells." *J. Bacteriol.* https://doi.org/10.1128/JB.00858-07

Flemming, H.C., Wingender, J., 2010. The biofilm matrix. *Nat. Rev. Microbiol*. https://doi.org/10.1038/nrmicro2415

Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: An emergent form of bacterial life. *Nat. Rev. Microbiol.* https://doi.org/10.1038/nrmicro.2016.94

Fletcher, J.T., Gaze, R.H., 2008. Mushroom Pest and Disease Control: A Color Handbook 192. https://books.google.fr/books?id=LiIZhzUYYX8C&pgis=1&redir_esc=y&hl=fr

Fortineau, N., Trieu-Cuot, P., Gaillot, O., Pellegrini, E., Berche, P., Gaillard, J.L., 2000. Optimization of green fluorescent protein expression vectors for *in vitro* and *in vivo* detection of *Listeria monocytogenes*. *Res. Microbiol.* 151, 353–360. https://doi.org/10.1016/S0923-2508(00)00158-3

FranceAgriMer, ANICC, 2017. Analyse et suivi concurrentiel du champignon de couche frais et transformé en Europe en 2016. Montreuil. http://www.franceagrimer.fr/content/download/54720/529404/file/SYN-FEL2017 Champignon de couche.pdf

FranceAgriMer, ANICC, 2015. Analyse et positionnement concurrentiel du champignon en Europe. http://www.franceagrimer.fr/content/download/37756/347093/file/SYN-FEL-2015 Présent Champ Couche.pdf

Fritze, D., 2004. Taxonomy of the Genus *Bacillus* and Related Genera: The Aerobic Endospore-Forming Bacteria. *Phytopathology* 94, 1245–1248. https://doi.org/10.1094/PHYTO.2004.94.11.1245

Glamočlija, J., Soković, M., Ljaljević-Grbić, M., Vukojević, J., Milenković, I., Van Griensven, L., 2008. Morphological characteristics and mycelial compatibility of different *Mycogone perniciosa* isolates. *J. Microsc.* 232, 489–492. https://doi.org/10.1111/j.1365-2818.2008.02145.x

Godfrey, S.A.C., Harrow, S.A., Marshall, J.W., Klena, J.D., 2001. Characterization by 16S rRNA Sequence Analysis of Pseudomonads Causing Blotch Disease of Cultivated *Agaricus bisporus*. *Appl. Environ. Microbiol.* 67, 4316–4323. https://doi.org/10.1128/AEM.67.9.4316-4323.2001

Gordon, R.E., Haynes, W.C., Pang, C.H.-N., 1973. The genus *Bacillus*, Handbook n. ed. *Agricultural Research Service*, Washington.

Grewal, S.I.S., Rainey, P.B., 1991. Phenotypic variation of *Pseudomonas putida* and *P. tolaasii* affects the chemotactic response to *Agaricus bisporus* mycelial exudate. *J. Gen. Microbiol.* 137, 2761–2768. https://doi.org/10.1099/00221287-137-12-2761

Guo, Q., Dong, W., Li, S., Lu, X., Wang, P., Zhang, X., Wang, Y., Ma, P., 2014. Fengycin produced by *Bacillus subtilis* NCD-2 plays a major role in biocontrol of cotton seedling damping-off disease. *Microbiol. Res.* 169, 533–540. https://doi.org/10.1016/j.micres.2013.12.001

Guthrie, J.L., Castle, A.J., 2006. Chitinase production during interaction of *Trichoderma aggressivum* and *Agaricus bisporus*. *Can. J. Microbiol*. 52, 961–967. https://doi.org/10.1139/w06-054

Harman, G.E., 2006. Overview of Mechanisms and Uses of *Trichoderma spp*. *Phytopathology* 96, 190–194. https://doi.org/10.1094/PHYTO-96-0190

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species - Opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*. https://doi.org/10.1038/nrmicro797

Henkels, M.D., Kidarsa, T.A., Shaffer, B.T., Goebel, N.C., Burlinson, P., Mavrodi, D. V., Bentley, M.A., Rangel, L.I., Davis, E.W., Thomashow, L.S., Zabriskie, T.M., Preston, G.M., Loper, J.E., 2014. *Pseudomonas protegens* Pf-5 Causes Discoloration and Pitting of Mushroom Caps Due to the Production of Antifungal Metabolites. *Mol. Plant-Microbe Interact.* 27, 733–746. https://doi.org/10.1094/MPMI-10-13-0311-R

Hermosa, M.R., Grondona, I., Monte, E., 1999. Isolation of *Trichoderma harzianum* Th2 from Commercial Mushroom Compost in Spain. *Plant Dis.* 83, 591. https://doi.org/10.1094/PDIS.1999.83.6.591B

Hobley, L., Ostrowski, A., Rao, F. V, Bromley, K.M., Porter, M., Prescott, A.R., MacPhee, C.E., van Aalten, D.M.F., Stanley-Wall, N.R., 2015. Correction for Hobley et al., BslA is a self-assembling bacterial hydrophobin that coats the *Bacillus subtilis* biofilm. *Proc. Natl. Acad. Sci.* 112, E5371-5. https://doi.org/10.1017/CBO9781107415324.004

Hobley, L., Ostrowski, A., Rao, F. V, Bromley, K.M., Porter, M., Prescott, A.R., MacPhee, C.E., van Aalten, D.M.F., Stanley-Wall, N.R., 2013. BslA is a self-assembling bacterial hydrophobin that coats the *Bacillus subtilis* biofilm. *Proc. Natl. Acad. Sci.* 110, 13600–13605. https://doi.org/10.1073/pnas.1306390110

Hubert, D., n.d. Les champignons de Passy : origine des champignons de Paris. http://mapage.noos.fr/hubert.demory/champignons.htm

IBMA France, 2017. Le marché du biocontrôle désormais mesuré tous les ans grâce au Baromètre d'IBMA France 1–2 https://www.ibmafrance.com/wp-content/uploads/2018/01/171019_CP_IBMA_France_MARCHE_BIOCONTROLE_2016.pdf.

Idris, E.E., Iglesias, D.J., Talon, M., Borriss, R., 2007. Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Mol. Plant-Microbe Interact.* 20, 619–626. https://doi.org/10.1094/MPMI-20-6-0619

Jarmer, H., Berka, R., Knudsen, S., Saxild, H.H., 2002. Transcriptome analysis documents induced competence of *Bacillus subtilis* during nitrogen limiting conditions. *FEMS Microbiol.* Lett. 206, 197–200. https://doi.org/10.1016/S0378-1097(01)00525-0

Jia, K., Gao, Y.H., Huang, X.Q., Guo, R.J., Li, S.D., 2015. Rhizosphere inhibition of cucumber *Fusarium* wilt by different surfactin-excreting strains of *Bacillus subtilis*. *Plant Pathol. J.* 31, 140–151. https://doi.org/10.5423/PPJ.OA.10.2014.0113

Jiang, J., Ding, L., Michailides, T.J., Li, H., Ma, Z., 2009. Molecular characterization of field azoxystrobin-resistant isolates of *Botrytis cinerea*. *Pestic. Biochem. Physiol*. 93, 72–76. https://doi.org/10.1016/j.pestbp.2008.11.004

Joshi, R., McSpadden Gardener, B.B., 2006. Identification and characterization of novel genetic markers associated with biological control activities in *Bacillus subtilis*. *Phytopathology* 96, 145–154. https://doi.org/10.1094/PHYTO-96-0145

Kaplan, J.B., 2010. Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. *J. Dent. Res.* https://doi.org/10.1177/0022034509359403

Kearns, D.B., Chu, F., Rudner, R., Losick, R., 2004. Genes governing swarming in *Bacillus subtilis* and evidence for a phase variation mechanism controlling surface motility. *Mol. Microbiol.* 52, 357–369. https://doi.org/10.1111/j.1365-2958.2004.03996.x

Kesel, S., Grumbein, S., Gümperlein, I., Tallawi, M., Marel, A.K., Lieleg, O., Opitz, M., 2016. Direct comparison of physical properties of *Bacillus subtilis* NCIB 3610 and B-1 biofilms. *Appl. Environ. Microbiol.* 82, 2424–2432. https://doi.org/10.1128/AEM.03957-15

Kim, P. Il, Ryu, J.-W., Kim, Y.H., Chi, Y.T., 2010. Production of biosurfactant lipopeptides iturin A, fengycin, and surfactin A from *Bacillus subtilis* CMB32 for control of *Colletotrichum gloeosporioides*. *J. Microbiol. Biotechnol.* 20, 138–145. https://doi.org/10.4014/jmb.0905.05007

Kobayashi, K., Iwano, M., 2012. BslA(YuaB) forms a hydrophobic layer on the surface of *Bacillus subtilis* biofilms. *Mol. Microbiol.* 85, 51–66. https://doi.org/10.1111/j.1365-2958.2012.08094.x

Konstantinidis, K.T., Tiedje, J.M., 2005. Towards a genome-based taxonomy for prokaryotes. *J. Bacteriol.* 187, 6258–6264. https://doi.org/10.1128/JB.187.18.6258-6264.2005

Koumoutsi, A., Chen, X.H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Vater, J., Borriss, R., 2004. Structural and Functional Characterization of Gene Clusters Directing Nonribosomal Synthesis of Bioactive Cyclic Lipopeptides in *Bacillus amyloliquefaciens* Strain FZB42, *in*: Journal of Bacteriology. *American Society for Microbiology Journals*, pp. 1084–1096. https://doi.org/10.1128/JB.186.4.1084-1096.2004

Krupke, O.A., Castle, A.J., Rinker, D.L., 2003. The North American mushroom competitor, *Trichoderma aggressivum* f. *aggressivum*, produces antifungal compounds in mushroom compost that inhibit mycelial growth of the commercial mushroom *Agaricus bisporus. Mycol. Res.* 107, 1467–1475. https://doi.org/10.1017/S0953756203008621

Kunst, F., Rapoport, G., 1995. Salt stress is an environmental signal affecting degradative enzyme synthesis in *Bacillus subtilis*. *J. Bacteriol.* 177, 2403–2407. https://doi.org/10.1128/jb.177.9.2403-2407.1995

La commission des Communautés européennes, 2007. Directive n° 2007/6/CE du 14/02/07 modifiant la directive 91/414/CEE du Conseil afin d'y inscrire les substances actives metrafenone, *Bacillus subtilis*, spinosad et thiamethoxam. *AIDA*. https://aida.ineris.fr/consultation_document/913

Lahlali, R., Peng, G., Gossen, B.D., McGregor, L., Yu, F.Q., Hynes, R.K., Hwang, S.F., McDonald, M.R., Boyetchko, S.M., 2013. Evidence that the biofungicide serenade (*Bacillus subtilis*) suppresses clubroot on *Canola via* antibiosis and induced host resistance. *Phytopathology* 103, 245–254. https://doi.org/10.1094/PHYTO-06-12-0123-R

Lahlali, R., Peng, G., McGregor, L., Gossen, B.D., Hwang, S.F., McDonald, M., 2011. Mechanisms of the biofungicide Serenade (Bacillus subtilis QST713) in suppressing clubroot. *Biocontrol Sci. Technol.* 21, 1351–1362. https://doi.org/10.1080/09583157.2011.618263

Largeteau, M.L., Savoie, J.-M., 2010. Microbially induced diseases of *Agaricus bisporus*: biochemical mechanisms and impact on commercial mushroom production. *Appl. Microbiol. Biotechnol.* 86, 63–73. https://doi.org/10.1007/s00253-010-2445-2

Largeteau, M.L., Savoie, J.M., 2008. Effect of the fungal pathogen *Verticillium fungicola*1on fruiting initiation of its host, *Agaricus bisporus*. *Mycol. Res.* 112, 825–828. https://doi.org/10.1016/j.mycres.2008.01.018

Les champignonnières, n.d. Histoire du champignon de Paris. http://ruedeslumieres.morkitu.org/apprendre/champignon/origine/index_origine.html

López, D., Kolter, R., 2010. Extracellular signals that define distinct and coexisting cell fates in *Bacillus subtilis*. *FEMS Microbiol.* Rev. 34, 134–149. https://doi.org/10.1111/j.1574-6976.2009.00199.x

López, D., Vlamakis, H., Losick, R., Kolter, R., 2009. Paracrine signaling in a bacterium. *Genes Dev*. 23, 1631–1638. https://doi.org/10.1101/gad.1813709

Lu, X.M., Lu, P.Z., 2018. Response of microbial communities to pesticide residues in soil restored with *Azolla imbricata*. *Appl. Microbiol. Biotechnol.* 102, 475–484. https://doi.org/10.1007/s00253-017-8596-7

Luan, F.G., Zhang, L.L., Lou, Y.Y., Wang, L., Liu, Y.N., Zhang, H.Y., 2015. Analysis of microbial diversity and niche in rhizosphere soil of healthy and diseased cotton at the flowering stage in southern Xinjiang. *Genet. Mol. Res.* 14, 1602–1611. https://doi.org/10.4238/2015.March.6.7

Luo, C., Liu, X., Zhou, H., Wang, X., Chen, Z., 2015. Nonribosomal peptide synthase gene clusters for lipopeptide biosynthesis in *Bacillus subtilis* 916 and their phenotypic functions. *Appl. Environ. Microbiol.* 81, 422–431. https://doi.org/10.1128/AEM.02921-14

Ma, X., Wang, X., Cheng, J., Nie, X., Yu, X., Zhao, Y., Wang, W., 2015. Microencapsulation of *Bacillus subtilis* B99-2 and its biocontrol efficiency against *Rhizoctonia solani* in tomato. *Biol. Control* 90, 34–41. https://doi.org/10.1016/j.biocontrol.2015.05.013

Mamoun, M.L., Savoie, J.M., Olivier, J.M., 2000. Interactions between the pathogen *Trichoderma harzianum* Th2 and *Agaricus bisporus* in mushroom compost. *Mycologia* 92, 233–240. https://doi.org/10.2307/3761556

McGee, C.F., 2018. Microbial ecology of the *Agaricus bisporus* mushroom cropping process. *Appl. Microbiol. Biotechnol.* 102, 1075–1083. https://doi.org/10.1007/s00253-017-8683-9

McGee, C.F., Byrne, H., Irvine, A., Wilson, J., 2017a. Diversity and dynamics of the DNA and cDNA-derived bacterial compost communities throughout the *Agaricus bisporus* mushroom cropping

process. *Ann. Microbiol.* 67, 751–761. https://doi.org/10.1007/s13213-017-1303-1
McGee, C.F., Byrne, H., Irvine, A., Wilson, J., 2017b. Diversity and dynamics of the DNA-and cDNA-derived compost fungal communities throughout the commercial cultivation process for *Agaricus bisporus. Mycologia* 109, 475–484. https://doi.org/10.1080/00275514.2017.1349498
Mehta, C.M., Gupta, V., Singh, S., Srivastava, R., Sen, E., Romantschuk, M., Sharma, A.K., 2013. Role of microbiologically rich compost in reducing biotic and abiotic stresses. *Microorg. Environ. Manag. Microbes Environ.* 113–134. https://doi.org/10.1007/978-94-007-2229-3_5
Mehta, C.M., Palni, U., Franke-Whittle, I.H., Sharma, A.K., 2014. Compost: Its role, mechanism and impact on reducing soil-borne plant diseases. *Waste Manag.* 34, 607–622. https://doi.org/10.1016/j.wasman.2013.11.012
Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Göker, M., 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14, 60. https://doi.org/10.1186/1471-2105-14-60
Menzies, J.G., 1993. A strain of *Trichoderma viride* pathogenic to germinating seedlings of cucumber, pepper and tomato. *Plant Pathol.* 42, 784–791. https://doi.org/10.1111/j.1365-3059.1993.tb01565.x
Mielich-Süss, B., Lopez, D., 2015. Molecular mechanisms involved in *Bacillus subtilis* biofilm formation. *Environ. Microbiol.* https://doi.org/10.1111/1462-2920.12527
Ministère de l'Agriculture de l'Agroalimentaire et de la Forêt, Ministère de l'Ecologie du Developpement durable et de l'Energie, 2016. Plan Ecophyto II - Agro-écologie, produisons autrement. *Agro-écologie Produisons Autrem.* 1–66.
Moyne, A.L., Shelby, R., Cleveland, T.E., Tuzun, S., 2001. Bacillomycin D: An iturin with antifungal activity against *Aspergillus flavus. J. Appl. Microbiol.* 90, 622–629. https://doi.org/10.1046/j.1365-2672.2001.01290.x
Mumpuni, A., Sharma, H.S.S., Brown, A.E., 1998. Effect of metabolites produced by *Trichoderma harzianum* biotypes and *Agaricus bisporus* on their respective growth radii in culture. *Appl. Environ. Microbiol.* 64, 5053–5056.
Munsch, P., Alatossava, T., Marttinen, N., Meyer, J.M., Christen, R., Gardan, L., 2002. *Pseudomonas costantinii* sp. nov., another causal agent of brown blotch disease, isolated from cultivated mushroom sporophores in Finland. *Int. J. Syst. Evol. Microbiol.* https://doi.org/10.1099/ijs.0.02090-0
Nicolas, V., n.d. Exploration et étude des cavités creusées ou aménagées par l'homme; Val de loire, réutilisation des carrières : les champignonières. http://troglos.free.fr/dossiers_val_de_loire /dossier_vdl_carriere_champi/dossier_chapitre_1.html.
Noble, R., Coventry, E., 2005. Suppression of soil-borne plant diseases with composts: A review. *Biocontrol Sci. Technol.* 15, 3–20. https://doi.org/10.1080/09583150400015904
Ntougias, S., Zervakis, G.I., Kavroulakis, N., Ehaliotis, C., Papadopoulou, K.K., 2004. Bacterial diversity in spent mushroom compost assessed by amplified rDNA restriction analysis and sequencing of cultivated isolates. *Syst. Appl. Microbiol.* 27, 746–754. https://doi.org/10.1078/0723202042369857
Oerke, E.-C., 2006. Crop losses to pests. *J. Agric. Sci.* 144, 31. https://doi.org/10.1017/S0021859605005708
Ongena, M., Jacques, P., 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiol.* https://doi.org/10.1016/j.tim.2007.12.009
Ongena, M., Jacques, P., Touré, Y., Destain, J., Jabrane, A., Thonart, P., 2005. Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of Bacillus subtilis. *Appl. Microbiol. Biotechnol.* 69, 29–38. https://doi.org/10.1007/s00253-005-1940-3
Ospina-Giraldo, M.D., Royse, D.J., Thon, M.R., Chen, X., Romanie, C.P., 1998. Phylogenetic relationships of *Trichoderma harzianum* causing mushroom green mold in Europe and North America to other species of *Trichoderma* sources from world-wide. *Mycologia* 90, 76–81. https://doi.org/10.2307/3761014
Otalvaro, J.O., Brigante, M., 2018. Interaction of pesticides with natural and synthetic solids. Evaluation in dynamic and equilibrium conditions. *Environ. Sci. Pollut. Res.* 25, 6707–6719.

https://doi.org/10.1007/s11356-017-1020-0
Özcengiz, G., Öğülür, I., 2015. Biochemistry, genetics and regulation of bacilysin biosynthesis and its significance more than an antibiotic. *N. Biotechnol.* https://doi.org/10.1016/j.nbt.2015.01.006
Palazzini, J.M., Dunlap, C.A., Bowman, M.J., Chulze, S.N., 2016. *Bacillus velezensis* RC 218 as a biocontrol agent to reduce *Fusarium* head blight and deoxynivalenol accumulation: Genome sequencing and secondary metabolite cluster profiles. *Microbiol. Res.* 192, 30–36. https://doi.org/10.1016/j.micres.2016.06.002
Parisot, J., Carey, S., Breukink, E., Chan, W.C., Narbad, A., Bonev, B., 2008. Molecular mechanism of target recognition by subtilin, a class I lanthionine antibiotic. *Antimicrob. Agents Chemother.* 52, 612–618. https://doi.org/10.1128/AAC.00836-07
Patel, H., Tscheka, C., Edwards, K., Karlsson, G., Heerklotz, H., 2011. All-or-none membrane permeabilization by fengycin-type lipopeptides from *Bacillus subtilis* QST713. *Biochim. Biophys. Acta - Biomembr.* 1808, 2000–2008. https://doi.org/10.1016/j.bbamem.2011.04.008
Pathak, K. V., Keharia, H., 2014. Identification of surfactins and iturins produced by potent fungal antagonist, *Bacillus subtilis* K1 isolated from aerial roots of banyan (*Ficus benghalensis*) tree using mass spectrometry. *3 Biotech* 4, 283–295. https://doi.org/10.1007/s13205-013-0151-3
Perugini, M., Tulini, S.M.R., Zezza, D., Fenucci, S., Conte, A., Amorena, M., 2018. Occurrence of agrochemical residues in beeswax samples collected in Italy during 2013–2015. *Sci. Total Environ.* 625, 470–476. https://doi.org/10.1016/j.scitotenv.2017.12.321
Punja, Z.K., Rodriguez, G., Tirajoh, A., 2016. Effects of *Bacillus subtilis* strain QST 713 and storage temperatures on post-harvest disease development on greenhouse tomatoes. *Crop Prot.* 84, 98–104. https://doi.org/10.1016/j.cropro.2016.02.011
Raaijmakers, J.M., de Bruijn, I., Nybroe, O., Ongena, M., 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiol. Rev.* https://doi.org/10.1111/j.1574-6976.2010.00221.x
Rainey, P.B., 1991. Effect of *Pseudomonas putida* on hyphal growth of *Agaricus bisporus*. *Mycol. Res.* 95, 699–704. https://doi.org/10.1016/S0953-7562(09)80817-4
Raza, W., Ling, N., Yang, L., Huang, Q., Shen, Q., 2016. Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. Sci. Rep. 6, 24856. https://doi.org/10.1038/srep24856
Richter, M., Rossello-Mora, R., 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci.* 106, 19126–19131. https://doi.org/10.1073/pnas.0906412106
Richter, M., Rosselló-Móra, R., Oliver Glöckner, F., Peplies, J., 2015. JSpeciesWS: A web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32, 929–931. https://doi.org/10.1093/bioinformatics/btv681
Roh, J.Y., Liu, Q., Choi, J.Y., Wang, Y., Shim, H.J., Xu, H.G., Choi, G.J., Kim, J.C., Je, Y.H., 2009. Construction of a recombinant *Bacillus velezensis* strain as an integrated control agent against plant diseases and insect pests. *J. Microbiol. Biotechnol.* 19, 1223–1229. https://doi.org/10.4014/jmb.0902.065
Romero, D., Aguilar, C., Losick, R., Kolter, R., 2010. Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proc. Natl. Acad. Sci.* 107, 2230–2234. https://doi.org/10.1073/pnas.0910560107
Rooney, A.P., Price, N.P.J., Ehrhardt, C., Sewzey, J.L., Bannan, J.D., 2009. Phylogeny and molecular taxonomy of the *Bacillus subtilis* species complex and description of *Bacillus subtilis* subsp. inaquosorum subsp. nov. *Int. J. Syst. Evol. Microbiol.* 59, 2429–2436. https://doi.org/10.1099/ijs.0.009126-0
Royse, D.J., Baars, J., Tan, Q., 2017. Current Overview of Mushroom Production in the World, *in:* Edible and Medicinal Mushrooms. *John Wiley & Sons*, Ltd, Chichester, UK, pp. 5–13. https://doi.org/10.1002/9781119149446.ch2
Samuels, G.J., 1996. *Trichoderma*: A review of biology and systematics of the genus. *Mycol. Res.* https://doi.org/10.1016/S0953-7562(96)80043-8
Samuels, G.J., Dodd, S.L., Gams, W., Castlebury, L.A., Petrini, O., 2002. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycologia* 94,

146–170. https://doi.org/10.1080/15572536.2003.11833257
Samuels, G.J., Petrini, O., Manguin, S., 1994. Morphological and macromolecular characterization of *Hypocrea schweinitzii* and its *Trichoderma* anamorph. *Mycologia* 86, 421–435. https://doi.org/10.2307/3760575
Saravanakumar, K., Yu, C., Dou, K., Wang, M., Li, Y., Chen, J., 2016. Synergistic effect of *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum*. *Biol. Control* 94, 37–46. https://doi.org/10.1016/j.biocontrol.2015.12.001
Savary, S., Ficke, A., Aubertot, J.N., Hollier, C., 2012. Crop losses due to diseases and their implications for global food production losses and food security. *Food Secur.* 4, 519–537. https://doi.org/10.1007/s12571-012-0200-5
Savoie, J.M., Iapicco, R., Largeteau-Mamoun, M.L., 2001. Factors influencing the competitive saprophytic ability of *Trichoderma harzianum* Th2 in mushroom (*Agaricus bisporus*) compost. *Mycol. Res.* 105, 1348–1356. https://doi.org/10.1017/S0953756201004993
Savoie, J.M., Mata, G., 2015. Growing *Agaricus bisporus* as a Contribution to Sustainable Agricultural Development, *in:* Mushroom Biotechnology: Developments and Applications. *Elsevier*, pp. 69–91. https://doi.org/10.1016/B978-0-12-802794-3.00005-9
Seaby, D.A., 1996. Investigation of the epidemiology of green mould of mushroom (*Agaricus bisporus*) compost caused by *Trichoderma harzianum*. *Plant Pathol.* 45, 913–923. https://doi.org/10.1111/j.1365-3059.1996.tb02902.x
Sharifi, R., Ryu, C.M., 2016. Are bacterial volatile compounds poisonous odors to a fungal pathogen *Botrytis cinerea*, alarm signals to *Arabidopsis* seedlings for eliciting induced resistance, or both? *Front. Microbiol*. 7, 196. https://doi.org/10.3389/fmicb.2016.00196
Singh, P.K., Lal, A., Sharma, S.K., Simon, S., 2013. Influence of *Pseudomonas putida* on the yield of *Agaricus bisporus* (Lange) Imbach. Internat. *J. Plant Protec*. 6, 2013.
Siwulski, M., Sobieralski, K., Górski, R., Lisiecka, J., Sas-Golak, I., 2011. Temperature and PH impact on the mycelium growth of *Mycogone perniciosa* and *Verticillium fungicola* isolates derived from Polish and foreign mushroom growing houses. *J. Plant Prot.* Res. 51, 268–272. https://doi.org/10.2478/v10045-011-0044-6
Soković, M., Van Griensven, L.J.L.D., 2006. Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus. Eur. J. Plant Pathol.* 116, 211–224. https://doi.org/10.1007/s10658-006-9053-0
Stanley, N.R., Lazazzera, B.A., 2005. Defining the genetic differences between wild and domestic strains of *Bacillus subtilis* that affect poly-γ-DL-glutamic acid production and biofilm formation. *Mol. Microbiol.* 57, 1143–1158. https://doi.org/10.1111/j.1365-2958.2005.04746.x
Stein, T., 2005. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. *Mol. Microbiol.* https://doi.org/10.1111/j.1365-2958.2005.04587.x
Szczech, M., Staniaszek, M., Habdas, H., Uliński, Z., SzymańSki, J., 2008. *Trichoderma spp.* – The cause of green mold on polish mushroom farms. *Veg. Crop. Res. Bull.* 69, 105–114. https://doi.org/10.2478/v10032-008-0025-0
Teeling, H., Meyerdierks, A., Bauer, M., Amann, R., Glöckner, F.O., 2004. Application of tetranucleotide frequencies for the assignment of genomic fragments. *Environ. Microbiol.* 6, 938–947. https://doi.org/10.1111/j.1462-2920.2004.00624.x
Teng, P. S. and Krupa, S. V., 1980. Assessment of losses which constrain production and crop improvement in agriculture and forestry, *in:* E. C. Stackman Commemorative Symposium. *Dept. of Plant Pathology*, University of Minnesota, p. 327.
Teng, P.S., 1987. Crop loss assessment and pest management, *in:* American Phytopathological Society. *American Phytopathological Society*, p. 270.
Tenoux, I., Besson, F., Michel, G., 1991. Studies on the antifungal antibiotics: bacillomycin D and bacillomycin D methylester. *Microbios* 67, 187–193.
Timmusk, S., Grantcharova, N., Wagner, E.G.H., 2005. *Paenibacillus polymyxa* invades plant roots and forms biofilms. *Appl. Environ. Microbiol.* 71, 7292–7300. https://doi.org/10.1128/AEM.71.11.7292-7300.2005

Tremblay, Y.D.N., Hathroubi, S., Jacques, M., 2014. Les biofilms bactériens: Leur importance en santé animale et en santé publique. Can. J. Vet. Res. 78(2): 110–116

Trieu-Cuot, P., Carlier, C., Poyart-Salmeron, C., Courvalin, P., 1991. Shuttle vectors containing a multiple cloning site and a *lacZ*α gene for conjugal transfer of DNA from *Escherichia coli* to Gram-positive bacteria. *Gene* 102, 99–104. https://doi.org/10.1016/0378-1119(91)90546-N

Uwizeyimana, H., Wang, M., Chen, W., Khan, K., 2017. The eco-toxic effects of pesticide and heavy metal mixtures towards earthworms in soil. *Environ. Toxicol. Pharmacol*. 55, 20–29. https://doi.org/10.1016/j.etap.2017.08.001

van Gestel, J., Vlamakis, H., Kolter, R., 2015. From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. *PLoS Biol.* 13, e1002141. https://doi.org/10.1371/journal.pbio.1002141

Vehmaanperä, J., 1989. Transformation of *Bacillus amyloliquefaciens* by electroporation. *FEMS Microbiol*. *Lett.* 61, 165–170. https://doi.org/10.1111/j.1574-6968.1989.tb03572.x

Verhamme, D.T., Kiley, T.B., Stanley-Wall, N.R., 2007. DegU co-ordinates multicellular behaviour exhibited by *Bacillus subtilis. Mol. Microbiol.* 65, 554–568. https://doi.org/10.1111/j.1365-2958.2007.05810.x

Viterbo, A., Ramot, O., Chernin, L., Chet, I., 2002. Significance of lytic enzymes from *Trichoderma spp.* in the biocontrol of fungal plant pathogens. Antonie van Leeuwenhoek, *Int. J. Gen. Mol. Microbiol.* 81, 549–556. https://doi.org/10.1023/A:1020553421740

Vlamakis, H., Aguilar, C., Losick, R., Kolter, R., 2008. Control of cell fate by the formation of an architecturally complex bacterial community. *Genes Dev.* 22, 945–953. https://doi.org/10.1101/gad.1645008

Vlamakis, H., Chai, Y., Beauregard, P., Losick, R., Kolter, R., 2013. Sticking together: Building a biofilm the *Bacillus subtilis* way. *Nat. Rev. Microbiol*. https://doi.org/10.1038/nrmicro2960

Wang, H., Fewer, D.P., Holm, L., Rouhiainen, L., Sivonen, K., 2014. Atlas of nonribosomal peptide and polyketide biosynthetic pathways reveals common occurrence of nonmodular enzymes. *Proc. Natl. Acad. Sci*. 111, 9259–9264. https://doi.org/10.1073/pnas.1401734111

Weissman, K.J., 2015. The structural biology of biosynthetic megaenzymes. *Nat. Chem. Biol*. https://doi.org/10.1038/nchembio.1883

Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot*. 52, 487–511. https://doi.org/10.1093/jexbot/52.suppl_1.487

Williams, J., Clarkson, J.M., Mills, P.R., Cooper, R.M., 2003. Saprotrophic and mycoparasitic components of aggressiveness of *Trichoderma harzianum* groups toward the commercial mushroom *Agaricus bisporus*. *Appl. Environ. Microbiol.* 69, 4192–4199. https://doi.org/10.1128/AEM.69.7.4192-4199.2003

Wu, Z., Hao, Z., Zeng, Y., Guo, L., Huang, L., Chen, B., 2015. Molecular characterization of microbial communities in the rhizosphere soils and roots of diseased and healthy Panax notoginseng. Antonie van Leeuwenhoek, *Int. J. Gen. Mol. Microbiol.* 108, 1059–1074. https://doi.org/10.1007/s10482-015-0560-x

Xu, Z., Zhang, R., Wang, D., Qiu, M., Feng, H., Zhang, N., Shen, Q., 2014. Enhanced control of cucumber wilt disease by *Bacillus amyloliquefaciens* SQR9 by altering the regulation of its DegU phosphorylation. *Appl. Environ. Microbiol.* 80, 2941–2950. https://doi.org/10.1128/AEM.03943-13

Yi, Y., Kuipers, O.P., 2017. Development of an efficient electroporation method for rhizobacterial *Bacillus mycoides* strains. *J. Microbiol. Methods* 133, 82–86. https://doi.org/10.1016/j.mimet.2016.12.022

Yoshida, N., Sato, M., 2009. Plasmid uptake by bacteria: A comparison of methods and efficiencies. *Appl. Microbiol. Biotechnol*. https://doi.org/10.1007/s00253-009-2042-4

Zhang, G.Q., Bao, P., Zhang, Y., Deng, A.H., Chen, N., Wen, T.Y., 2011. Enhancing electro-transformation competency of recalcitrant *Bacillus amyloliquefaciens* by combining cell-wall weakening and cell-membrane fluidity disturbing. *Anal. Biochem.* 409, 130–137. https://doi.org/10.1016/j.ab.2010.10.013

Zhang, N., Yang, D., Wang, D., Miao, Y., Shao, J., Zhou, X., Xu, Z., Li, Q., Feng, H., Li, S., Shen, Q.,

Zhang, R., 2015. Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates. *BMC Genomics* 16, 685. https://doi.org/10.1186/s12864-015-1825-5

Zhao, X., Han, Y., Tan, X. qian, Wang, J., Zhou, Z. jiang, 2014. Optimization of antifungal lipopeptide production from *Bacillus sp.* BH072 by response surface methodology. *J. Microbiol.* 52, 324–332. https://doi.org/10.1007/s12275-014-3354-3

Zhao, X., Kuipers, O.P., 2016. Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. *BMC Genomics* 17, 882. https://doi.org/10.1186/s12864-016-3224-y

VALORISATIONS SCIENTIFIQUES

Formations effectuées au sein de l'Unité Maiage de Jouy en Josas:

Traitement sous Galaxy de données NGS Miseq 16SrDNA et ITS, Assemblage et annotation génome issue de séquençage Hiseq :

- Métagénomique bioinformatique sous Galaxy
- RNA-Seq bioinformatique sous Galaxy
- Statistiques sous « R » & RNA-Seq

Congrès, colloques, séminaires, journées:

- 2ème colloque bactéries sporulantes ou d'intérêt technologique (BSIPT 2018) – Paris, 27 Mars 2018
- 8ème colloque du Réseau National Biofilm – Clermont-Ferrand, 5 & 6 Décembre 2017
- VIIIe Colloque de l'Association Francophone d'Ecologie Microbienne (AFEM) – Camaret-sur-mer, 17 - 20 Octobre 2017.
- 7ème symposium Doc'Micalis 2017 – Jouy-en-Josas, 31 Mars 2017
- Journée doctorale DIM ASTREA 2017 – Paris, 25 Mars 2016
- ISMS Congress - International Society for Mushroom Science – Amsterdam, 29-02 Juin 2016
- Journées doctorale ABIES – Paris, 14 & 15 Avril 2016
- 4ème séminaire du DIM ASTREA sur l'agriculture biologique en Ile de France – Paris, 29 Mars 2016
- Journée doctorale DIM ASTREA 2016 – Paris, 25 Mars 2016
- 7ème colloque Réseau National Biofilm – Toulouse, 2 & 3 Décembre 2015

Communications orales:

- C. Pandin, D. Le Coq, J. Deschamps, R. Vedie, T. Rousseau, S. Aymerich, R. Briandet. (2018). *Bacillus velezensis* QST713 pour la bioprotection des champignons de Paris. 2ème colloque bactéries sporulantes ou d'intérêt technologique (BSIPT) – Paris 27 mars 2018. **Prix de la meilleure communication orale.**
- C. Pandin, D. Le Coq, S. Aymerich, R. Briandet. (2017). Mechanisms involved in the bioprotection of the button mushroom *Agaricus bisporus* by *Bacillus subtilis* biofilms. 7ème symposium Doc'Micalis – Jouy-en-Josas – 2 juin 2017
- C. Pandin, D. Le Coq, S. Aymerich, R. Briandet. (2017). Mécanismes impliqués dans la bioprotection des champignons de Paris, *Agaricus bisporus*, par les biofilms de *Bacillus subtilis*. Journée doctorale DIM ASTREA – Paris, 31 Mars 2017

Posters:

- C. Pandin, J. Deschamps, D. Le Coq, A. Canette, S. Aymerich, R. Briandet. (2017) Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? 8ème colloque du Réseau National Biofilm – Clermont-Ferrand, 5 & 6 Décembre 2017
- C. Pandin, R. Védie, T. Rousseau, S. Aymerich, D. Le Coq, R. Briandet (2017) Evolution of compost microbial communities submitted or not to the presence of *Bacillus subtilis* QST713 and/or *Trichoderma aggressivum* during cultivation of *Agaricus bisporus*. VIIIe Colloque de l'Association Francophone d'Ecologie Microbienne (AFEM) – Camaret-sur-mer, 17 - 20 octobre 2017
- C. Pandin, R. Védie, T. Rousseau, A. Canette, J. Deschamps, S. Aymerich, D. Le Coq, R. Briandet (2016) *Bacillus subtilis* biofilm for the bioprotection of the cultivated button mushrooms. Biofilm 7 International Conference – Porto, 26 au 28 Juin 2016

- C. Pandin, R. Védie, T. Rousseau, A. Canette, J. Deschamps, S. Aymerich, D. Le Coq, R. Briandet (2016) Bioprotection of the cultivated mushroom Agaricus bisporus by Bacillus subtilis biofilms.
    - ISMS 2016 - Amsterdam - 29 Mai au 3 juin 2016.
    - 4ème séminaire du DIM sur la recherche en agriculture biologique en Île-de-France le 29 mars 2016 à Paris.
    - Journée doctorale DIM ASTREA - Paris - 25 Mars 2016

Encadrement:

- Encadrement stagiaire M1 – Janvier 2016 à Juin 2016 : stage, suivi et correction de rapport, représentant soutenance.

Publications scientifiques liées à ce travil de thèse:

Articles publiés:

- Pandin, C, Le Coq, D, Canette, A, Aymerich, S, Briandet, R. (2017). Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? Microb. Biotechnol. 10, 719–734. https://doi.org/10.1111/1751-7915.12693
- Bridier, A, Piard, J-C, Pandin, C, Labarthe, S, Dubois-Brissonnet, F, Briandet, R. (2017). Spatial Organization Plasticity as an Adaptive Driver of Surface Microbial Communities. Front. Microbiol. 8:1364. https://doi.org/10.3389/fmicb.2017.01364
- Pandin, C, Le Coq, D, Deschamps, J, Védie, R, Rousseau, T, Aymerich, S, Briandet, R. (2018) Complete genome sequence of *Bacillus velezensis* QST713: A biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease. J Biotech. 278:10-19. https://doi.org/10.1016/j.jbiotec.2018.04.014
- Pandin, C., Védie, R., Rousseau, T., Le Coq, D., Aymerich, S., & Briandet, R. (2018). Dynamics of compost microbiota during the cultivation of *Agaricus bisporus* in the presence of *Bacillus velezensis* QST713 as biocontrol agent against *Trichoderma aggressivum*. Biological Control, 127, 39–54. https://doi.org/10.1016/J.BIOCONTROL.2018.08.022

Article en cours d'écriture:

- Pandin, C, Darsonval, M, Mayeur, C, Le Coq, D, Aymerich, S, Briandet, R. Biofilm formation and antimicrobial synthesis by the biocontrol agent Bacillus velezensis QST713 in Agaricus bisporus compost micromodel.

Valorisations diverses:

- Diffusion sur le site pleinchamp.com d'un article vulgarisé. 27 Juillet 2017. « Le biofilm, nouveau protecteur d'une agriculture écologique » publié par l'INRA de Jouy-en-Josas de la review « Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? ». http://www.pleinchamp.com/actualites-generales/actualites/le-biofilm-nouveau-protecteur-d-une-agriculture-ecologique%20/
- Séminaire INRA/Institut Français de Presse - Conférence de Presse - Le biofilm, nouveau protecteur d'une agriculture écologique - 23/25 Janvier 2018. Médiation scientifique auprès d'étudiants journalistes lors du séminaire.
- Dossier de presse INRA grand public – Les conquêtes de l'Inra pour le biocontrôle. 11 Juillet 2018. http://presse.inra.fr/Communiques-de-presse/Les-conquetes-de-l-Inra-pour-le-biocontrole. https://inra-dam-front-resources-cdn.brainsonic.com/ressources/afile/444595-12ba9-resource-dp-biocontrole.pdf, page 19.

-

université PARIS-SACLAY
ÉCOLE DOCTORALE
Agriculture, alimentation, biologie, environnement, santé (ABIES)

Titre : Exploration des mécanismes impliqués dans la bioprotection d'*Agaricus bisporus* par les biofilms de *Bacillus subtilis* QST713.

Mots clés : *Bacillus velezensis*, Biofilm, Biocontrol, *Agaricus bisporus*, Microbiote compost, *Trichoderma aggressivum*

Résumé : Les pertes alimentaires mondiales se chiffrent à environ un tiers des aliments destinés à la consommation humaine, soit environ 1,3 milliards de tonnes par an (FAO). Une large fraction de ces pertes est due aux altérations microbiologiques des denrées alimentaires. L'utilisation de produits phytosanitaires reste aujourd'hui la solution la plus largement utilisée en agriculture pour limiter ces pertes. Cependant, avec le plan EcoPhyto 2, le gouvernement français a pour objectif de réduire de 50% l'usage des pesticides chimiques d'ici 2025, en particulier en promouvant l'émergence du biocontrôle. Pour développer cette approche, il est cependant nécessaire de comprendre, pour mieux les maitriser, les mécanismes sous-jacents. Les différents modes d'action de biocontrôle par les microorganismes décrits sont la stimulation des défenses naturelles des plantes, la production de substances antimicrobienne et la compétition nutritionnelle. L'originalité de ce projet est d'intégrer le mode de vie en biofilm dans les mécanismes de bioprotection (compétition spatiale et nutritionnelle, libération de principes antimicrobiens). Dans la filière Française des champignons de couche (*Agaricus bisporus*), l'agent de biocontrôle utilisé depuis 2008 par plus de 80 % de la filière, est *Bacillus subtilis* QST713. Ce biofongicide montre une nette efficacité contre *Trichoderma aggressivum*, la principale moisissure à l'origine de pertes économiques lors de la culture d'*A. bisporus*. Afin d'accompagner la filière dans cette voie biologique, nous avons entrepris de séquencer et étudier le génome de cette souche, afin de déterminer son potentiel de biocontrôle et sa capacité à former des biofilms. Nous avons également évalué l'impact de ce biofongicide sur la dynamique des communautés microbiennes du compost de culture d'*A. bisporus* exposé ou non à *T. aggressivum*. Enfin, l'étude de la reprogrammation cellulaire de cet agent de biocontrôle lors de sa culture en micromodèles axéniques, nous a permis une meilleure compréhension des phénomènes de colonisation des substrats et d'inhibition des flores indésirables. Ce projet a permis d'enrichir les connaissances vis-à-vis des mécanismes de biocontrôle dans la filière des champignons et pourra permettre une possible application à d'autres filières agricoles.

Title : Exploration of mecanisms involved in the bioprotection of *Agaricus bisporus* by *Bacillus subtilis* QST713 biofilms.

Keywords : *Bacillus velezensis*, Biofilm, Biocontrol, *Agaricus bisporus*, Compost microbiota, *Trichoderma aggressivum*

Abstract : Worldwide, food losses amount for about one-third of food for human consumption, 1.3 billion tons per year (FAO). A large fraction of these losses is due to microbiological alterations. The use of phytosanitary products remains today the most widely used solution in agriculture to limit these losses. However, with the EcoPhyto 2 plan, the French government aims to reduce the use of chemical pesticides by 50% by 2025, in particular by promoting the emergence of biocontrol. To develop this approach, it is necessary to understand the underlying mechanisms. The different modes of action of biocontrol by the microorganisms described are the stimulation of the natural defenses of the plants, the production of antimicrobial substances and the nutritional competition. The originality of this project is to integrate the biofilm mode of life into bioprotection mechanisms (spatial and nutritional competition, release of antimicrobial principles). In the French sector of the button mushrooms (*Agaricus bisporus*) culture, the biocontrol agent used since 2008 by more than 80% of the sector, is *Bacillus subtilis* QST713.This biofungicide shows a clear efficacy against *Trichoderma aggressivum*, the main mold causing economic losses during the cultivation of *A. bisporus*. To accompany the sector in this biological pathway, we have sequenced and studied the genome of this strain, in order to determine its biocontrol potential and its ability to form biofilms. We also evaluated the impact of this biofungicide on the dynamics of microbial communities in *A. bisporus* culture compost exposed or not to *T. aggressivum*. Finally, the study of the cellular reprogramming of this biocontrol agent during the culture in axenic micromodels allowed us a better understanding of the substrates colonization phenomenon and the inhibition of undesirable flora. This project enriched the knowledge of the biocontrol mechanisms used in the mushroom industry and may allow a possible application to other agricultural sectors.

Université Paris-Saclay
Espace Technologique / Immeuble Discovery
Route de l'Orme aux Merisiers RD 128 / 91190 Saint-Aubin, France