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Genetic diversity and pectinolytic activity of epiphytic yeasts from grape carposphere

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ABSTRACT. The genetic diversity of epiphytic yeasts from grape carposphere is susceptible to environmental variations that determine the predominant carposphere microbiota. Understanding the diversity of yeasts that inhabit grape carposphere in different environments and their pectinolytic activity is a way to understand the biotechnological potential that surrounds us and help improve winemaking. Therefore, this study aimed to evaluate the pectinolytic activity and characterize the genetic diversity of isolated epiphytic yeasts from grape carposphere. Grapes of the Bordeaux cultivar were collected from different regions of Paraná and Rio Grande do Sul States, in Brazil, and the yeasts were isolated from these grape carpospheres. Monosporic isolates were morphologically and genetically characterized on potato dextrose agar medium and by PCR-RFLP and rep-PCR (BOX-PCR) in the ITS1-5.8S-ITS2 region of rDNA. The index of pectinolytic activity of isolates was also evaluated estimating the ratio between the halo diameter of enzymatic degradation and the diameter of the colony when the isolates

were grown in cultivation medium containing 10 g/L pectin, 5 g/L yeast extract, 15 g/L agar, 0.12% (w/v) Congo red, and pH 6.2. We observed that the grape carposphere is an environment with a great genetic diversity of epiphytic yeasts of the following genera: *Cryptococcus* (31.25%), *Pichia* (25.0%), *Candida* (25.0%), *Dekkera* (12.5%), and *Saccharomyces* (6.25%). The PCR-RFLP technique allowed analyzing existing polymorphism among individuals of a population based on a more restrict and evolutionarily preserved region, mostly utilized to differentiate isolates at the genus level. Approximately 33% of yeast isolates presented pectinolytic activity with potential biotechnological for wine and fruit juice production. This great genetic variability found indicated that it is a potential reservoir of genes to be applied in viniculture improvement programs.

Key words: Biotechnology; Yeast; Taxonomy; BOX-PCR; PCR-RFLP; Ribosomal DNA

INTRODUCTION

The first grapevine varieties cultivated in Brazil were *Vitis vinífera* L., introduced by the Portuguese, and *Vitis labrusca* L., introduced by the Italian. Among *V. labrusca*, Bordeaux cultivar has stood out for its capacity to adapt to Brazilian climatic conditions, high productivity, and tolerance to fungal diseases (Rizzon et al., 2000). It also stands out for the production of a must with intense color and high acidity. This cultivar is mainly utilized for the production of juices and fine wines (Pommer, 2003).

Several factors are involved in the production of high-quality wine such as the grape cultivar, the cultivation environment, culture management, wild yeast strains involved in the fermentation process besides the yeast strain used in winemaking. Yeast is the responsible agent for the transformation of must sugar into alcohol during the fermentation process to produce wine, producing and releasing pectinases that with β-glucanases and hemicellulases help the extraction, clearing, and stabilization of wine, resulting in improvement in its quality and stability (Bhat, 2000; Takayanagi et al., 2001). According to Gump and Halght (1995), pectinases reduce winemaking costs due to the elimination and/or substitution of enhancers in grape processing and winemaking, increasing productivity.

Winemaking is a complex process that involves the sequential development of different yeast species (Sabate et al., 2002) mostly found in grape carposphere. The surface of ripe grapes has a microbial community that varies from 10^3 to 10^5 CFU/g, and most of them are yeasts and lactic and acetic bacteria (Fleet, 1999). As soon as a grape is crushed and its content is released, the natural fermentation process occurs due to yeasts that are naturally found in grape carposphere. The population of yeasts related with grape carposphere depends on grape variety, its maturation level, and grapevine geographical location (Sabate et al., 2002). According to Sabate et al. (2002), yeasts that are found in grape carposphere are the predominant microbiota of the must with development in the first stages of the fermentation process, having an important role in fermentation and quality of the final product. Therefore, it is essential to identify and characterize yeasts found on the grape carposphere to obtain better quality wine (Combina et al., 2005).

The development of molecular techniques for DNA analysis such as PCR (polymerase chain reaction) and DNA sequencing allowed establishing strategies for more accurate taxonomic identification of microbial isolates (Barcellos and Hungria, 2010). For fungi, in general, including yeasts, the rDNA region (ITS1-5.8S-ITS2) has been utilized for the taxonomic identification of the genus and species through PCR-RFLP (Restriction Fragment Length Polymorphism) (Barcellos and Hungria, 2010). Despite having been developed for repetitive elements of prokaryotic genomes, the PCR of repetitive elements (rep-PCR) is also an identification option that has been utilized for eukaryotic genomes and in studies of diversity and taxonomy of yeasts (Loncaric et al., 2009).

The knowledge of pectinolytic yeast populations found in grape carposphere is relevant for the occurrence of a good fermentation process and a better quality of the final product. Thus, in this study, we characterize the genetic diversity and evaluate the pectinolytic activity of isolated epiphytic yeasts from grape carposphere of Bordeaux cultivar.

MATERIAL AND METHODS

Grape harvest and yeast isolation

Grapes (*Vitis labrusca* L.) of Bordeaux cultivar were harvested from five grape-producing regions of Paraná State: Colombo, Marialva, Miqueleto, Santa Felicidade - Curitiba, and Warta - Londrina, and from six regions of Rio Grande do Sul State: Bento Gonçalves, Caxias do Sul, Farroupilha, Garibaldi, Pinto Bandeira, and Veranópolis, Brazil, and stored in a refrigerator. Whole grapes, without damages, were washed with autoclaved (121°C for 30 min) distilled water under agitation for 250 rpm, for 30 min, at 25°C. Aliquots of 0.1 mL washing water were spread on potato dextrose agar medium (PDA). The isolated yeasts were purified until obtaining a pure culture, confirmed by visualization under an optical microscope and cryopreserved at -80°C in culture medium containing Czapek-Dox broth (CBD) and glycerol (1:1). All isolated yeasts were deposited in the culture collection of the Department of General Biology of Londrina State University.

Characterization of genetic diversity of isolated yeasts

Morphological characterization

Colonies of isolated yeasts were grown on PDA medium for 72 h at 28°C and grouped according to the shape, size, elevation, color, consistency, and aspect of the margin of the colonies. The cell structures were observed under optical microscope and colonies were photographed and separated by different morphotypes.

Extraction of genomic DNA

A sample of each morphotype was selected for DNA extraction and molecular analysis. Each isolate was cultivated in YPD (10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose) cultivation media for 3 days, washed in autoclaved (121°C for 30 min) distilled water, and centrifuged at 12,000 *g*. The formed pellet was re-suspended in 200 µL lyse buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0), 200

μL phenol:chloroform:isoamyl alcohol (25:24:1) solution, and homogenized in a Vortex agitator with glass beads (Ausubel et al., 2002). After, 200 μL Tris:EDTA (10:1), pH 8.0, was added and centrifuged at 12,000 *g* for 5 min. The aqueous phase was transferred to a new tube with 1 mL cold absolute ethanol, centrifuged at 2000 *g*, washed with 70% ethanol, centrifuged at 2000 *g*, air dried at room temperature and solubilized in 50 μL ultrapure water. The concentration and quality of extracted DNA were evaluated by electrophoresis on agarose gel at 0.8% (w/v) in 0.5X TBE buffer (Tris-Borate-EDTA) at 5 V/cm for 90 min. The gels were stained with ethidium bromide (0.5 mg/mL), according to Sambrook and Russell (2006), and the images were digitalized using an L-PIX EX photo documenter (Loccus Biotecnologia) under ultraviolet (UV) light.

PCR

Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTA TTGATATGC-3') were utilized to amplify the ITS1-5.8S-ITS2 region of rDNA. The mixture of PCR (25 μL) consisted of PCR 1X buffer (Life Technologies), 0.2 mM dNTP mixture, 1.5 mM $MgCl_2$, 0.5 pmol of each primer, 1.25 U *Taq* DNA polymerase (Life Technologies) and 10 ng genomic DNA of each isolate. PCR was carried out in a thermocycler (Amplitherm Thermal Cyclers) with an initial denaturation cycle for 5 min at 95°C, followed by 35 cycles of amplification (94°C 1 min, 56°C 1 min, 72°C 1 min) and a final extension cycle for 5 min at 72°C. To visualize the amplification products, 1 μL final PCR product was submitted to electrophorese on 1% agarose gel (w/v) using the molecular mass marker of 1 kb plus DNA ladder (Life Technologies) and ethidium bromide (Sambrook and Russell, 2006). In all PCR reactions a tube containing all reagents, except for DNA sample, was utilized as a negative control.

Restriction of amplification products of the ITS1-5.8S-ITS2 region of ribosomal DNA (PCR-RFLP)

The enzymes utilized in the restriction analysis of the ITS1-5.8S-ITS2 region were *Hin*fI, *Cfo*I, and *Hae*III. The digestion reaction (15 μL) consisted of 1.5 μL 10X digestion buffer, 3 U enzyme, 2 μL purified PCR product and ultrapure water to complete the volume. The digestions were done in a thermocycler (Amplitherm Thermal Cyclers) at 37°C for 90 min. The digestion product was applied in electrophorese gel with 2% agarose (w/v), using the molecular mass marker of 1 kb plus DNA ladder (Invitrogen) with ethidium bromide (Sambrook and Russell, 2006).

DNA amplification by rep-PCR

The primers described by Versalovic et al. (1991) were utilized for BOX-PCR (rep-PCR). PCR was carried out in a thermocycler (Amplitherm Thermal Cyclers) with a reaction volume of 30 μL PCR mix RED*Taq* (20 mM Tris-HCl, 100 mM KCl, 3 mM $MgCl_2$, 0.4 mM DNTP, 0.9 U *Taq* DNA polymerase, and 24 pM primers for BOX-PCR). In DNA amplifications, initial denaturation (7 min) occurred at 95°C, followed by 30 denaturation cycles (1 min at 94°C), pairing for 1 min at 53°C and extension (8 min at 65°C) with a final extension of 16 min at 65°C. The PCR product (15 μL) was submitted to electrophorese on 1.5% agarose gel (w/v) with molecular mass marker of 1 kb plus DNA ladder (Invitrogen) with ethidium bromide (Sambrook and Russell, 2006).

Dendrogram graph

Based on band profiles obtained from rep-PCR and PCR-RFLP amplifications of the ITS1-5.8S-ITS2 region of rDNA, isolates were grouped by DendroUPGMA program (http://genomes.urv.cat/UPGMA/; Garcia-Vallvé et al., 1999). A dendrogram construction was done utilizing UPGMA (unweighted pair-group method with arithmetic mean) algorithm (Sneath and Sokal, 1973) and Jaccard's coefficient at a tolerance level of 3% (Barcellos et al., 2007).

Pectinolytic activity of isolated yeasts

The isolates were cultivated for 3 days at 28°C on PDA and transferred to cultivation medium consisting of 10 g/L pectin, 5 g/L yeast extract, 15 g/L agar, and 0.12% (w/v) Congo red, pH 6.2, for 10 days at 28°C (Akbar and Prasuna, 2012). Pectinolytic activity was evaluated adding 3 mL 1 M HCl solution to the cultivation medium. The visualization of light halos around the colonies indicated substrate hydrolysis by pectinolytic activity. The enzymatic activity index was estimated by the ratio between the degradation halo and the colony diameter (Hankin and Anagnostakis, 1975). Assays were done in triplicate and reported as arithmetic average and standard deviation.

RESULTS AND DISCUSSION

Characterization of genetic diversity of isolated yeasts

Morphological characterization of isolated yeasts

Isolated yeasts (90) were morphologically characterized as *Saccharomyces* and non-*Saccharomyces* genera. Morphotypes presented differentiations in shape, texture, and shine of colonies with white, salmon, cream, or orange (Figure 1), and shiny or opaque (Figure 2) chromogenesis. The isolates were grouped based on morphological aspects in 18 morphologically distinct groups. A representative of each of these groups was selected for morphological analysis of the colony (Table 1), and a great morphological variability was observed, mainly in the regions of Colombo and Farroupilha (Figure 3).

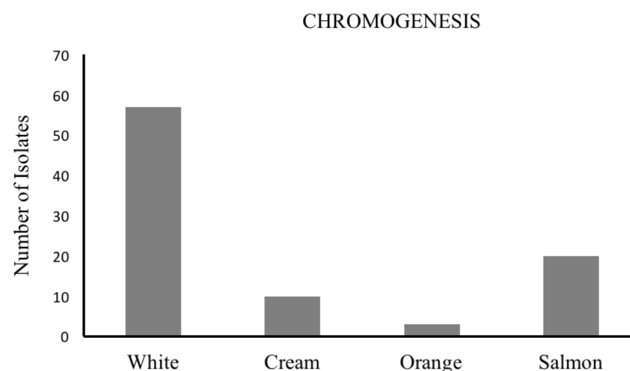

Figure 1. Chromogenesis of isolated yeast colonies of grape carposphere.

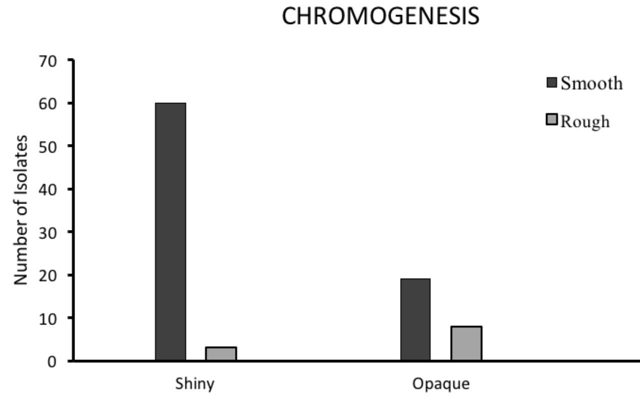

Figure 2. Optical detail and surface of isolated yeast colonies of grape carposphere.

Table 1. Morphological description of isolated yeasts from grape carposphere, based on macroscopic observations.

Isolate	Morphology of colonies						
	Form	Elevation	Margin	Chromogenesis	Optical detail	Surface	Consistency
Ub01	Irregular	Flat	Undulate	Salmon	Shiny	Smooth	Mucoid
Ub02	Irregular	Flat	Undulate	White	Opaque	Smooth	Dry
Ub03	Irregular	Flat	Undulate	White	Shiny	Smooth	Mucoid
Ub04	Circular	Flat	Entire	White	Opaque	Smooth	Mucoid
Ub05	Irregular	Raised	Lobate	White	Shiny	Rough	Gummy
Ub06	Circular	Flat	Undulate	White	Shiny	Smooth	Mucoid
Ub07	Circular	Flat	Entire	White	Shiny	Smooth	Mucoid
Ub08	Rhizoid	Flat	Undulate	Cream	Shiny	Smooth	Mucoid
Ub09	Irregular	Flat	Undulate	Salmon	Opaque	Rough	Dry
Ub10	Irregular	Convex	Lobate	Salmon	Shiny	Smooth	Gummy
Ub11	Irregular	Flat	Lobate	White	Opaque	Smooth	Dry
Ub12	Irregular	Flat	Undulate	White	Opaque	Smooth	Gummy
Ub13	Irregular	Umbonate	Lobate	White	Opaque	Rough	Powdery
Ub14	Circular	Flat	Entire	Salmon	Shiny	Smooth	Mucoid
Ub16	Circular	Convex	Entire	Salmon	Shiny	Smooth	Gummy
Ub17	Rhizoid	Flat	Filiform	Orange	Opaque	Rough	Dry
Ub18	Rhizoid	Flat	Filiform	White	Opaque	Smooth	Dry
Ub22	Circular	Flat	Entire	Cream	Shiny	Smooth	Mucoid

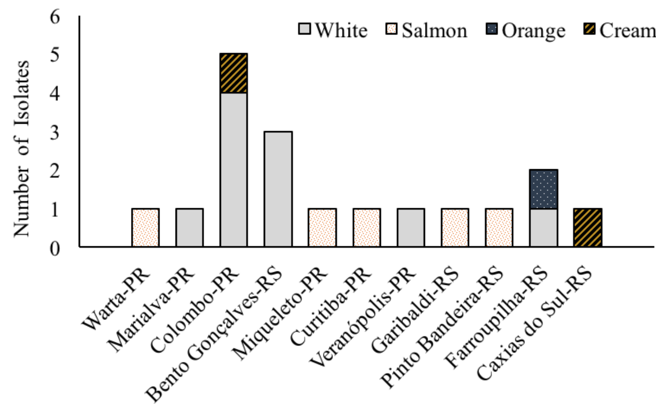

Figure 3. Number of isolated yeasts and morphotypes (colony color) collected from grape carposphere in different regions of Paraná (PR) and Rio Grande do Sul (RS) States, in Brazil.

Phenotypical characters and the number of yeasts can be affected by cultivation conditions, cultivar, harvesting period, climatic conditions, geographic location, stage of fruit ripening, the presence of physical damages, and the utilization of agricultural pesticides in grapes (Mortimer and Polsinelli, 1999); this can partly explain the greater morphological variability observed in the isolated yeasts. Moreover, the morphological variation makes yeast characterization by morphological characteristics difficult; therefore, the molecular technique becomes the most appropriate technique for a more accurate taxonomic classification (Tofalo et al., 2009).

Molecular (taxonomic) identification of yeasts

According to the morphological cluster, a representative isolate of each morphotype was selected for the taxonomic identification (genus). Based on the amplification of the ITS1-5.8S-ITS2 region of rDNA, the amplicons ranged from 425 to 850 bp. The fragments amplified by PCR (Figure 4) were digested with *Cfo*I, *Hin*fl, and *Hae*III restriction enzymes and the generated band profiles are shown in Table 2. These patterns were compared to the ones described by Esteve-Zarzoso et al. (1999).

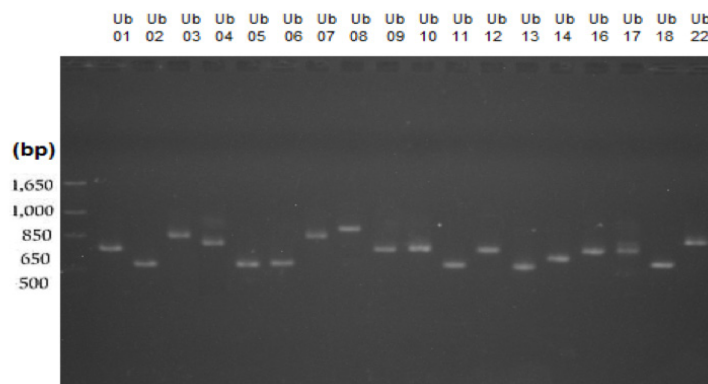

Figure 4. Amplicons (bp) of the ITS1-5.8S-ITS2 region of rDNA obtained by PCR of isolated yeasts of grape carposphere.

Table 2. Size (bp) of PCR and PCR-RFLP products and identification of isolated yeast genera of grape carposphere.

Genus	Isolate code	ITS* (bp)	Fragments of enzymatic restriction		
			*Cfo*I	*Hae*III	*Hin*fI
Cryptococcus	Ub01	660	345 + 315	400 + 70 + 70 + 60 + 60	370 + 290
Pichia	Ub02	500	175 + 110 + 90 + 75	330 + 90 + 50	275 + 200
Dekkera	Ub03	800	340 + 340 + 120	800	360 + 190 + 160 + 80
Candida	Ub05	580	510	370 + 190	290 + 260
Candida	Ub06	580	510	370 + 190	290 + 260
Dekkera	Ub07	800	340 + 340 + 120	375 + 95	270 + 215
Saccharomyces	Ub08	880	385 + 365	500 + 220 + 145	365 + 155
Cryptococcus	Ub09	630	330 + 300	500 + 70 + 60	350 + 280
Cryptococcus	Ub10	630	330 + 300	500 + 70 + 60	350 + 280
Pichia	Ub11	500	260 + 180 + 60	500	270 + 230
Candida	Ub13	425	400	425	225 + 200
Candida	Ub14	550	300 + 240	400 + 115	290 + 260
Cryptococcus	Ub16	630	330 + 300	500 + 70 + 60	350 + 280
Cryptococcus	Ub17	630	630	505 + 65 + 60	235 + 150 + 135 + 110
Pichia	Ub18	500	260 + 180 + 60	500	270 + 230
Pichia	Ub22	650	575	600 + 50	310+310

*Amplicon size obtained by PCR of the ITS1-5.8S-ITS2 region of ribosomal DNA.

According to Skinner et al. (1980) and Phaff (1990), grape carposphere consists mainly of yeast-like fungi such as *Aureobasidium*, *Rhodotorula*, *Cryptococcus*, *Candida*, *Pichia*, *Hanseniaspora*, and *Saccharomyces* genera, similar to most genera found in our study (Table 2). Other authors also cite the presence of *Saccharomyces cerevisiae*, *Candida valida*, *Kloeckera apiculata* and *Pichia membranifaciens* on the grape carposphere (Mamede and Pastore, 2004; Fernandes et al., 2008).

Pichia genus was found at the rate of 25% of isolates (Table 3). This genus contributed to the increase in wine aroma by synthesizing enzymes such as β-glucosidase (Maicas and Mateo, 2005). Lopes et al. (2009) found this genus in the initial stages of the grape fermentation process and consider it one of the responsible for the organoleptic properties of wine.

Table 3. Genus of isolated yeasts found in grape carposphere and respective isolation frequencies.

Traits	P	G
Direct effect of NNB on Y	0.070	-0.379
Indirect effect via NBN	0.136	0.921
Indirect effect via NP2	0.119	0.501
Indirect effect via NP3	0.034	0.053
Indirect effect via TNP	0.070	-0.528
Total	0.432	0.5683
Direct effect of NBN on Y	0.204	1.183
Indirect effect via NNB	0.047	-0.295
Indirect effect via NP2	0.127	0.492
Indirect effect via NP3	0.115	0.216
Indirect effect via TNP	0.115	-0.86
Total	0.6107	0.7324
Direct effect of NP2 on Y	0.288	1.039
Indirect effect via NNB	0.029	-0.183
Indirect effect via NBN	0.090	0.559
Indirect effect via NP3	0.055	-0.237
Indirect effect via TNP	0.117	-0.768
Total	0.5808	0.4099
Direct effect of NP3 on Y	0.534	1.123
Indirect effect via NNB	0.004	-0.018
Indirect effect via NBN	0.044	0.228
Indirect effect via NP2	0.029	-0.220
Indirect effect via TNP	0.931	0.467
Total	0.7061	0.6454
Direct effect of TNP on Y	0.163	-1.101
Indirect effect via NNB	0.030	-0.182
Indirect effect via NBN	0.144	0.928
Indirect effect via NP2	0.206	0.725
Indirect effect via NP3	0.304	0.477
Total	0.8494	0.8472
Determination coefficient	0.83	0.86
Residual effect	0.40	0.36

The most abundant genus observed in our study was *Cryptococcus* representing 31.25% of isolates (Table 3) and characterized by encapsulated yeasts that form pigmented colonies.

Candida genus was identified in 25% of isolates (Table 3). Yeasts of this genus have a potential for alcoholic fermentation of pentose (Du Preez et al., 1986); moreover, some species have an antifungal activity that controls wine contamination by filamentous fungi (Fleet, 2003).

Approximately 12% of isolates belong to *Dekkera* genus (Table 3). According to Esteve-Zarzoso et al. (1999), *Dekkera bruxellensis* is among the species that cause the greatest impact on wine quality by producing non-desirable aromatic compounds and is isolated from grape carposphere or in the wood barrel during the storage or aging of wines.

Saccharomyces genus, as reported by other authors (Martini, 1993; Pretorius, 2000), was observed at low frequency, only 6.25% (Table 3). According to Barata et al. (2012), this yeast, despite being very utilized in fermentation processes of wine production, is isolated in a smaller number when a direct isolation technique is used, suggesting that it is less frequent in grape carposphere. According to Fleet (1999), the number of fermentative yeasts such as *Saccharomyces* spp is greater when the grape skin is damaged, releasing its content to the grape carposphere, resulting in a population increase of 10^6 to 10^8 CFU/g when compared to undamaged grapes.

Analysis of genetic diversity of isolated yeast based on dendrograms obtained by BOX-PCR and PCR-RFLP in the ITS1-5.8S-ITS2 region of rDNA

BOX-PCR genomic marker differentiated nine groups (A, B, C, D, E, F, G, H, I) and two subgroups (B_1 and B_2) (≥50% similarity) (Figure 5). Group A was the only representative of Warta region (Londrina - PR), from *Cryptococcus* genus, and that presented colonies with salmon chromatogenesis and mucoid consistency. Group B, consisting of *Pichia* genus, presented two subgroups (Figure 5). B_1 was isolated in the region of Paraná State and, B_2 in the region of Rio Grande do Sul State. The chromatogenesis presented in the group colony was white with a dry consistency. The similarity among the subgroups was 60%. Group C, represented by *Saccharomyces* genus, was the only one from Colombo - PR and whose colony chromatogenesis had cream color with a mucoid consistency (Figure 5). Group D, consisting of two representatives of *Cryptococcus* genus, had a similarity of 66.7% and was from Curitiba - PR. They presented salmon chromatogenesis, but the colonies were different on the surface: Ub09 was rough and Ub10 was smooth. Group E (Figure 5), represented by *Pichia* genus, showed cream color chromatogenesis with mucoid consistency and was from Caxias do Sul - RS. Group F, represented by *Cryptococcus* genus (Ub16), was different from the other representatives of this genus (Ub01, Ub09, Ub10, Ub17) because its colonies had white chromatogenesis with mucoid consistency. Group G, consisting of *Candida* genus, with 75% of similarity, were all from Bento Gonçalves - RS, and had white colonies, differentiating themselves only by the consistency: UB05 was mucoid and Ub13 was dry (Figure 5 and Table 1). Group H, represented by *Candida* genus (Figure 5 and Table 1), with a single isolate from Garibaldi - RS, had colonies with salmon chromatogenesis and dry consistency. Group I, *Cryptococcus* genus, was the only isolate from Farroupilha - RS and presented colonies with orange chromatogenesis and dry consistency.

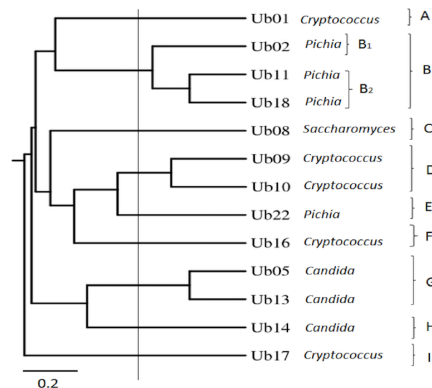

Figure 5. Cluster by BOX-PCR with analysis from the level at 50% similarity of isolated yeasts of grape carposphere.

The BOX-PCR technique, due to polymorphism generated in several regions of the genome, allowed differentiating genotypes. The PCR-RFLP technique in the ITS1-5.8S-ITS2 region analyzed the existing polymorphism among individuals of a population based on a more restricted and evolutionarily preserved region, which allowed the differentiation of isolates at the genus or superior taxonomic levels (Barcellos and Hungria, 2010). In our study, it was observed that most of the obtained clusters were formed by only one or two isolates, showing a high genetic diversity. Also, the isolated yeasts belonging to each group were from distinct regions where the grape was collected.

The analysis of restriction of the ITS1-5.8S-ITS2 region by the PCR-RFLP technique allowed observing the formation of nine groups (I, II, III, IV, V, VI, VII, VIII, IX) (Figure 6) with similarity level over 80%. Similarly to the clusters obtained by BOX-PCR, most of the formed groups consisted of only one of two isolates. In groups V and VI, the isolates were grouped at 100% similarity, and they were from *Pichia* and *Cryptococcus* genera, respectively.

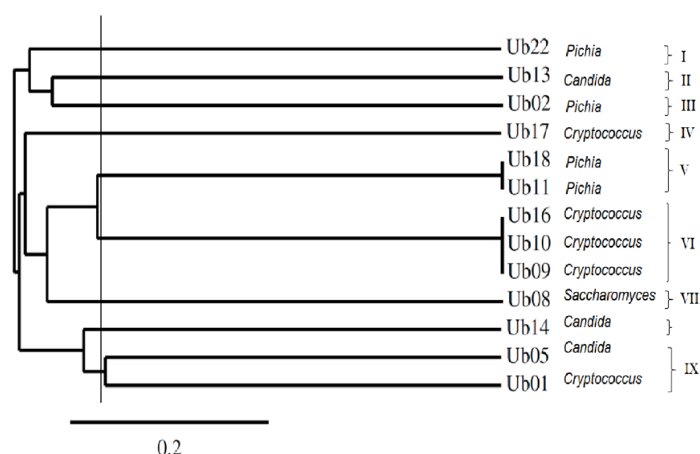

Figure 6. Cluster by PCR-RFLP with analysis from the level at 80% similarity of isolated yeasts of grape carposphere.

Comparing the cluster formed by BOX-PCR and PCR-RFLP, six groups presented the same similarity indexes, except for groups II, III, and IX that presented significant similarities. Therefore, it was possible to verify that isolated yeasts presented a broad genetic diversity, which potentially implies in potential future use for bioprospecting studies.

The exclusive utilization of morphological, biological, and physiological criteria to identify microbial communities is questionable due to their heterogeneous phenotypical results. The reproducibility of these methodologies is questionable as a result depends on the physiological state of the cell at that moment (Barata et al., 2012). However, despite being non-conclusive, these techniques can help and complement molecular techniques of identification. Molecular techniques directly analyze the individual genome, regardless of the physiological state of the cell, which allows a greater accuracy in the identification (Fernández-Espinar et al., 2011; Barata et al., 2012).

Our results indicated that the grape carposphere has a great variety of yeast species that, according to Pretorius et al. (1999), can spontaneously ferment sugars and generate

ethanol, carbon dioxide, and other metabolites, and can contribute to change the quality of the final product. Several factors such as temperature can affect yeast diversity on the grape carposphere (Rementeria et al., 2003). This genetic variability is a potential genetic reservoir for yeast breeding programs for winemaking, which contribute to the quality of the final product such as the aroma (Pretorius, 2004).

Pectinolytic activity

The isolated yeasts in our study presented potential to produce pectinases, and 33% of them presented enzymatic activity index over 2.0 (Table 3), which, according to Lealem and Gashe (1994), would be the minimum value to consider a microorganism an enzyme producer. Pectinases are utilized in several biotechnological areas, mainly in the food industry for maceration of vegetal tissues (Lea, 1995), extraction, and clarification of juices and wines (Hoondal et al., 2002). They are responsible for the hydrolysis of the cell wall releasing anthocyanins, which are pigments that confer a reddish color of fruits, and that are widely utilized in the production of red wine with reduction of maceration time of grape bark in the must (Kashyap et al., 2001).

CONCLUSION

The grape carposphere is an environment with a great genetic diversity of yeasts of *Cryptococcus* (31.25%), *Pichia* (25.0%), *Candida* (25.0%), *Dekkera* (12.5%), and *Saccharomyces* (6.25%) genera. PCR-RFLP technique allowed analyzing the polymorphism among individuals of a population based on a more restricted and evolutionarily preserved region and utilized mostly to differentiate isolates at the genus level. Most of the isolated yeasts (33%) present pectinolytic activity with potential biotechnological application in the production of wines and fruit juices. The great genetic variability of isolates indicates a potential genetic reservoir for viniculture improvement programs.

Conflicts of interest

The authors declare no conflict of interest.

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