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Biotransformation of grape pomace from *Vitis labrusca* by *Peniophora albobadia* LPSC # 285 (Basidiomycota)

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Abstract: Grape pomace from Vitis labrusca is an important sub-product of the "American table wine" industry. It is recalcitrant to degradation, and its accumulation is a serious problem with negative environmental impacts. We analyzed the ability of five white-rot fungi to transform this residue in-vitro. Mass loss and phenol removal in grape pomace treated with each fungus were compared after 30-day solid-state fermentation. Since *Peniophora albobadia* isolate LPSC 285 was the fungus that showed the highest degradative ability and the lowest free phenol levels in the residue transformed, we selected this fungus to monitor its effect on this residue after 30, 60, and 90 days of incubation. We analyzed mass loss of the residue caused by the fungus activity and its chemical changes using Fourier transform infrared spectroscopy. After 90 days of incubation, *Peniophora albobadia* isolate LPSC 285 reduced grape pomace mass by 20.48%, which was associated with degradation of polysaccharides and aromatic structures. We concluded that *Peniophora albobadia* LPSC # 285 isolate is a promising fungus to transform grape pomace from Vitis labrusca under solid-state fermentation conditions.

Key words: fungi, grape pomace, *Peniophora albobadia*, transformation, *Vitis labrusca*.

INTRODUCTION

"Cooperativa de la Costa" (Buenos Aires, Argentina) is a small enterprise aimed at producing craft red wine from *Vitis labrusca* var. Isabella (L) grapes in eastern Buenos Aires province, Argentina (Velarde et al. 2013). This wine was recognized by the National Institute of Vitiviniculture as a regional product with proper sensory features, produced by traditional manufacturing procedures (Resolution No. 23; http://www.loa.org.ar/legNormaDetalle.aspx?id=23547, Velarde et al. 2013). However, it generates large amounts of organic debris during a short period of time (February and March of each year). This debris is accumulated over long storage intervals without any treatment. This debris accumulation leads to deleterious environmental impacts and phytosanitary risks. One of its most important residues is grape pomace (GP), which is composed of skins and seeds as well as remaining grape pulp. A typical composition of GP is 10.3% cellulose, 12.0% hemicellulose, 37.2% lignin, and a high percentage of moisture (< 75%). Such sub-products might be used to improve soils by incorporating organic matter and its nutrients for plant growth. However, the skin and seeds contained in this organic waste have a low

pH and high levels of phenolic compounds, which are toxic to microorganisms and might also prevent plant growth (Bustamante et al. 2008, Rockenbach et al. 2011). Ferrer et al. (2001), Díaz et al. (2002), Burg et al. (2014), and Dominguez et al. (2014) transformed debris from wine production by processes such as composting and vermicomposting, which stabilize this organic matter, and used it for an agronomic application. However, they represent complex biological processes demanding long processing periods, during which earthworms are highly sensitive to pH, temperature as well as moisture and phenolic compound content (Rostami 2011). Furthermore, while the studies about the revalorization of grape pomace are from different varieties of *V. vinifera*, no studies are available on GP of *V. labrusca*.

White rot fungi (WRF) are known for their ability to enhance degradation and/or detoxification of a wide variety of agricultural or agro-industrial wastes under solid-state fermentation (SSF), e.g. wheat straw, rice straw, olive mill residue (alpeorujo), and post-harvest sugarcane (Dorado et al. 1999, Maza et al. 2014). The ability of these fungi to transform organic wastes relies on their capacity to release nonspecific oxidative enzymes such as laccases and peroxidases. These enzymes can oxidize and transform lignin and a wide range of structurally similar compounds (Saparrat et al. 2002, 2010b). This biodegradation might be used to treat GP and convert it into a by-product capable of improving soil conditions, among other applications. To date not much information has been made available regarding fungal transformation of GP (Sanchez et al. 2002, Botella et al. 2007, Vallejo et al. 2012). Therefore, biodegradation of GP of *V. labrusca* under SSF conditions using WRF is lacking. The aim of this study was to evaluate the feasibility of five selected WRF to degrade GP and reduce its free phenol content. In addition, the transformation of GP by *Peniophora albobadia* LPSC 285 (Basidiomycota) was evaluated after 30, 60, and 90 days under SSF conditions.

MATERIALS AND METHODS

Grape pomace

It was collected directly from a basket press available at "Cooperativa de la Costa", an enterprise located in the city of Berisso (Buenos Aires Province, Argentina; latitude 34°53'22.79"S and longitude 57°49'21.11"O). All the fresh material collected during the harvest of 2008 (in March) was dried at 60 °C and representative aliquots of individual samples corresponding to several pressing procedures were used to form a composite pool sample. The material collected was stored for up to 6 months at 25 °C in a room with moisture levels as low as possible and then used as the substrate for growing the fungi.

Fungal isolates

Five WRF were used, three belonging to the *Aurantiporus* genus (two isolates of *A. albidus* and one of *A. pulcherrimus*), a representative of *P. albobadia* and another of *Pycnoporus sanguineus* (Table I). Fungal stock cultures were maintained as slants on malt extract agar (MEA) medium at 4 °C. Mycelium plugs (6-mm in diameter) cut from a culture grown on MEA medium were used as inoculum.

Mass loss and phenolic removal in GP by Fungi

For SSF, 100 mL Erlenmeyer Flasks containing 10 g of GP (dry mass) and 7 mL distilled water were sterilized at 121 ºC for 2 periods of 15 min. Flasks were inoculated with three mycelium plugs and incubated at 28 ± 1.5 ºC at 70% relative humidity for 30 days. Uninoculated control flasks were incubated under the same conditions. The experimental design was completely randomized

Table I. White-rot fungi used in this study.

Species	Isolate	Reference
Aurantiporus pulcherrimus (Rodway) P.K. Buchanan & Hood	CIEFAP[a] 80	Hood et al. 2008
Aurantiporus albidus Rajchenb. & Cwielong	CIEFAP 111	Rajchenberg and Robledo 2013
Aurantiporus albidus Rajchenb. & Cwielong	CIEFAP 117	Rajchenberg and Robledo 2013
Pycnoporus sanguineus (L:Fr.) Murr.	LPSC[b] 163	Saparrat et al. 2002
Peniophora albobadia (Schw.: Fr.) Boidin	LPSC 285	Saparrat et al. 2002

[a]Mycological herbarium at Centro Forestal Andino Patagónico. Chubut, Argentina. [b]Culture collection of Instituto Spegazzini, La Plata. Buenos Aires, Argentina.

with three replicates per treatment. Percentage of mass reduction of inoculated GP in relation to the uninoculated one was assessed according to Saparrat et al. (2010a). A water soluble fraction (WSF) from uninoculated and inoculated GP was obtained according to Saparrat et al. (2008), and its phenolic concentration was estimated (Saparrat et al. 2010a).

Time course of GP transformation by *P. albobadia* LPSC 285

The fungus was cultivated on GP under SSF conditions as described above for 30, 60, and 90 days of incubation. Uninoculated sterilized GP was incubated under the same conditions and used as an abiotic control. The experimental design was completely randomized, and three independent replicates were carried out for each time of incubation, with a solid fraction (SF) and a WSF obtained from both uninoculated and fungal transformed GP, according to Saparrat et al. (2008). On SFs, the percentage of mass reduction of inoculated GP in relation to the uninoculated one was estimated, and its chemical composition was studied using Fourier transform infrared (FT-IR) spectroscopy, according to Saparrat et al. (2010a). Decay rate (k) of GP, both inoculated and uninoculated, was estimated using an exponential decay model (Mallerman et al. 2018). In the WSF of uninoculated and inoculated GP, concentration of ammonium-nitrogen (NH_4^+-N; Saparrat et al. 2010a), total soluble reducing carbohydrates (TSRC; Saparrat et al. 2008), pH, and phenolics as well as the polymerization/polydispersity index (the ratio of optical density at 465 nm to that at 665 nm; Dorado et al. 1999) were determined. Also, the activity of β-1,4 endoglucanase (E.C. 3.2.1.4), laccase (EC 1.10.3.2) and peroxidase (EC 1.11.1.7) on the WSF obtained from fungal treated GP was determined. The activity of the β-1,4-endoglucanase was estimated by measuring the amount of reducing cellooligosaccharides released from sodium-carboxy-methylcellulose (BDH Chemicals Ltd; Saparrat et al. 2008). Laccase activity was measured using 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (Sigma-Aldrich) according to Saparrat et al. (2008). Peroxidase activity was estimated using 2,6-dimethoxyphenol (Fluka) as substrate in the presence of 0.1 mM H_2O_2 (Saparrat et al. 2008).

FT-IR Spectroscopy

For sample preparation, each SF from GP treated with *P. albobadia* for 30, 60, and 90 days and the corresponding uninoculated GP as well as an additional one at time zero (immediately after the sterilization) were dried at 60 °C. Each sample was homogenized with an agate mortar and sieved through < 0.63 mm screen. Each

resultant powder was embedded in KBr 13-mm pellets (2 mg of sample and 20 mg of infrared-grade KBr). FTIR absorption/transmission (A/T) spectra between 4,000 and 650 cm^{-1} were acquired using a Thermo Nicolet Nexus 470 spectrometer (Thermo-Electric Corporation, Chicago, IL, USA) with 4 cm^{-1} spectral resolution and 64 scan co-additions. To avoid interference of spectral water vapor bands, the spectra were measured under continuous purge of dried air. Two technical replicates were performed for each sample. Before data analysis, the whole set of spectra was subjected to a spectral pre-processing procedure. Briefly, technical replicates were averaged, and first and second derivatives were calculated using the Savitzky-Golay algorithm with 13-point smoothing and then vector normalized in the full spectral range using OPUS software (versions 7.0 Bruker Optics GmbH, Ettlingen, Germany; Naumann 2000). Bands selected as index peaks reflecting functional groups associated with the cell walls were assigned (Table II). The peak area at 904 cm^{-1} (A_{904}), which was assigned to the β-glycosidic content, and the peak area at 1262 cm^{-1} (A_{1262}), which was assigned to skeletal ring vibrations of lignin and used as an internal standard of the total biomass content, were calculated (Naumann 2000). The semi-quantitative evaluation of the β-glycosidic bond along the GP treatment was calculated according to the equation $\alpha = A_{904}/A_{1262}$ (OPUS software, versions 7.0 Bruker Optics GmbH, Ettlingen, Germany).

Statistical analysis

Data corresponding to mass loss and phenolic removal in GP by fungi as well as enzyme levels of *P. albobadia* LPSC 285 during culture time were analyzed by means of a one-way analysis of variance (ANOVA). The means of the treatments were contrasted using the Tukey's Test ($p \leq 0.05$; Statistix 8.0). Values of the A_{904}/A_{1262} ratio were

Table II. Assignment of FT-IR bands corresponding to functional groups.

Window Spectral regions (cm^{-1})	FTIR frequency (cm^{-1})	Assignment[a]	References
	3010	C-H def. in the plane of the aromatic ring in lignin	Stuart & Ando 1997
	2958	C-H asym. str. of $-CH_3$ in fatty acid chains	Stuart & Ando 1997, Naumann 2000
W1 Lipids 3,000-2,800	2924	C-H asym. str. of $>CH_2$ in fatty acid chains	Stuart & Ando 1997, Naumann 2000
	2873	C-H sym. str. of $-CH_3$ in fatty acids	Stuart & Ando 1997, Naumann 2000
	2853	C-H sym. str. of $>CH_2$ in fatty acids	Stuart & Ando 1997, Naumann 2000
W2 Amide I and II 1,800 -1,480	1744 1715	C=O str. of $COOCH_3$ and COOH groups in pectin	Batsoulis et al. 2004, Kyomugasho et al. 2015, Leão et al. 2018
	Amide I 1651	>C=O str. and C–N ben. of protein and peptides amide. Sensitive to protein conformation	Stuart & Ando 1997, Batsoulis et al. 2004
	Amide II 1540	N–H ben., C–N str. of proteins and peptides	Stuart & Ando 1997, Batsoulis et al. 2004

Table II (continuation)

Window Spectral regions (cm^{-1})	FTIR frequency (cm^{-1})	Assignment[a]	References
W3 Mixed Region 1,480 -1,250	1470 1450	CH_2 sym. scissoring of pyran ring in hemicellulose and cellulose	Schwanninger et al. 2004, Popescu et al. 2010, Heredia-Guerrero et al. 2014
	1416	CH_2 scissoring of cellulose	Schwanninger et al. 2004, Carballo-Meilan et al. 2014
	1377	CH_3 def. in the plane of carbohydrates	Pandey & Pitman 2003, Boeriu et al. 2004, Schwanninger et al. 2004, Popescu et al. 2010
	1340	C-O str. of syringyl unit of lignin	Pandey & Pitman 2003, Popescu et al. 2010, Saparrat et al. 2010a
	1310	CH_2 wagging of cellulose	Schwanninger et al. 2004, Carballo-Meilan et al. 2014
	1264	C-O str. of guaiacyl unit of lignin	Pandey & Pitman 2003, Popescu et al. 2010, Saparrat et al. 2010a
	1241	C-O str. of phenols groups of aromatics rings in syringyl and guaiacyl groups	Popescu et al. 2010
W4 Carbohydrates 1,200-900 900-700	1135	C-H def. in the plane in the guaiacyl ring	Kacuráková et al. 2000, Schwanninger et al. 2004
	1117	C-C and C-O str. in pectin	Batsoulis et al. 2004, Kyomugasho et al. 2015, Leão et al. 2018
	1106	COH def. in the plane in hemicellulose and cellulose	Boeriu et al. 2004, Schwanninger et al. 2004, Popescu et al. 2010
	1076 1066	C-O-C vibrations of arabinogalactan and glucomannan of hemicellulose	Kacuráková et al. 2000
	1033	C-H def. in the plane of the aromatic ring in lignin	Stuart & Ando 1997
	904	α-glycosidic linkages	Kacuráková et al. 2000, Synytsya et al. 2003, Popescu et al. 2010
	877	C-H def. in the plane of the aromatic ring in lignin	Dokken et al. 2002
	842	β-glycosidic linkages	Kacuráková et al. 2000, Synytsya et al. 2003, Popescu et al. 2010
	789	C–OH ring str. of pectin	Batsoulis et al. 2004, Kyomugasho et al. 2015, Leão et al. 2018

Table II (continuation)

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	1076 1066	C-O-C vibrations of arabinogalactan and glucomannan of hemicellulose	Kacuráková et al. 2000
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[a](asym.) asymmetric; (sym.) symmetric; (str.) stretching; (ben.) bending; (def.) deformation.

analyzed by one-way ANOVA, and a Fisher's least significant difference (LSD) procedure was applied to compare means ($p < 0.05$). A two-way nested ANOVA with two main effects, ''treatment type" (uninoculated GP versus inoculated GP) and ''incubation time", was applied to several physico-chemical parameters measured on the WSF obtained from GP untreated and treated with *P. albobadia* LPSC 285 at different incubation times. A Principal Component Analysis (PCA) was performed using PyChem software (versions 3.0.5g Beta) and, as input data, the first derivative of vector normalized data in the spectral regions 3000-2800, 1500-1250 and 1200-750 cm^{-1} (Naumann 2000, Jarvis et al. 2006).

RESULTS

Although all fungi reduced the GP mass and its phenolic content, *P. albobadia* LPSC 285 showed the highest degradative ability (Fig. 1). During 90 incubation days, this fungus reduced the GP mass by 20.48% to a rate of 0.002 $g.day^{-1}$, compared to that of the uninoculated residue (Fig. 2).

To further study the effect of *P. albobadia* LPSC 285 on GP, we monitored the transformations that took place in the residue over time (30, 60, 90 days) by A/T FTIR spectroscopy. Figure 3 shows absorbance FTIR spectra of fungal treated and untreated GP at time zero (immediately after the sterilization) and their corresponding second derivative vector normalized spectra at four main spectral windows. These windows

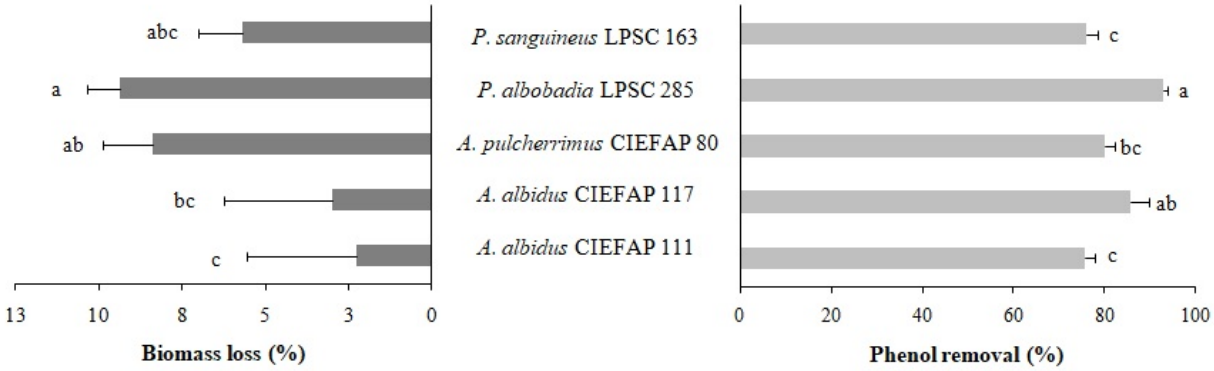

Figure 1. Biomass loss and phenol removal (%) of GP by WRF at 30 days. Means ± SD (bars) followed by the same letter are not significantly different according to Tukey's test, $p < 0.05$.

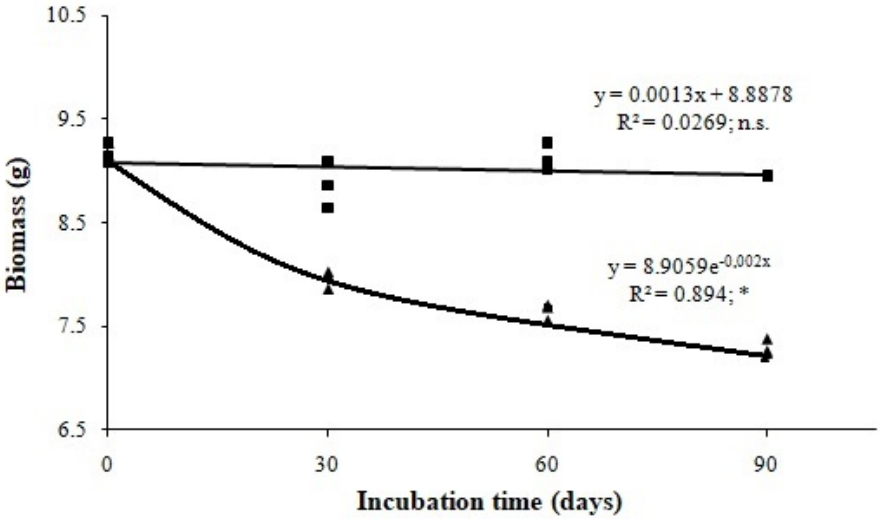

Figure 2. Regression analysis between biomass of GP uninoculated (■) and inoculated (▲) with *Peniophora albobadia* LPSC 285 and incubation time. Linear regression equation, F test significance (p) and coefficients of determination (R^2) are presented. (ns) Not significant; (*) $p < 0.05$.

were as follows: W1 (2800-3000 cm^{-1}), associated with lipids and presenting bands assigned to the anti-symmetric and symmetric stretching of $>CH_3$ detected at 2958 and 2873 cm^{-1}, respectively, and the anti-symmetric and symmetric stretching modes for the $>CH_2$ observed at 2924 and 2853 cm^{-1}, respectively; W2 (1800–1480 cm^{-1}), representing the typical amide I band at 1651 cm^{-1}, mainly due to >C=O absorptions, and amide II bands at 1540 cm^{-1}, assigned to the N––H bending with contributions of the C––N stretching vibrations in proteins; W3 (1480–1250 cm^{-1}), a mixed region presenting spectral peaks from C-H deformation (1377 cm^{-1}) and C-O stretching (1264, 1241 and 1215 cm^{-1}) corresponding to carbohydrates, as well as >C-O and >P=O symmetric stretching corresponding to fatty acid, proteins and lipids; and W4 (1250–900 cm^{-1}), showing bands that hold the peaks assigned to carbohydrate modes and side groups C-O-C, C-C and C-O.

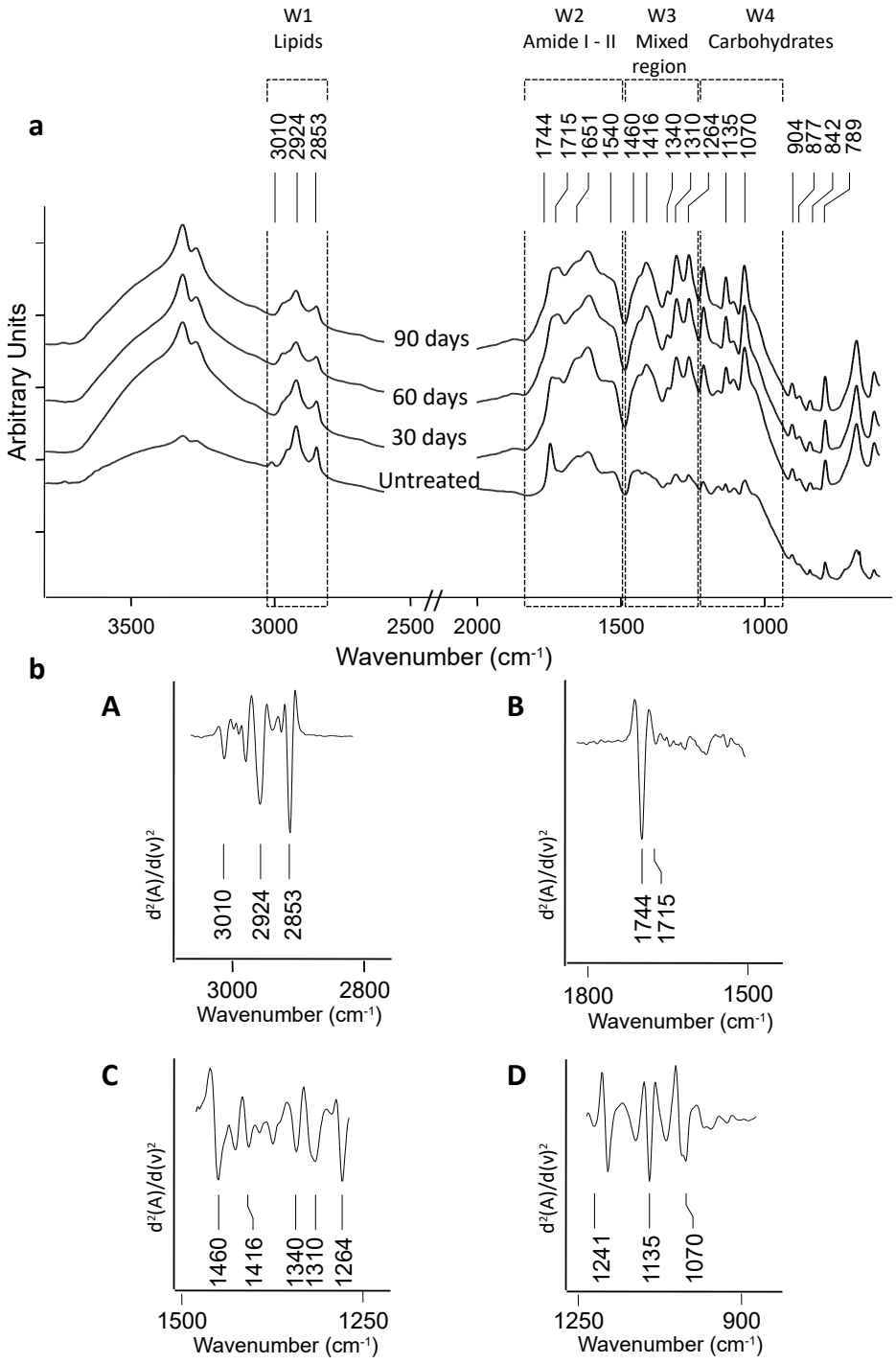

Figure 3. FT-IR absorption spectra of GP untreated and treated with *P. albobadia* LPSC 285 at several incubation times. **(a)** Four average spectra obtained from three independent experiments corresponding to the GP treated with *P. albobadia* LPSC 285 at several incubation times (30, 60, and 90 days) and uninoculated GP at time zero of incubation (immediately after the sterilization). Spectral windows associated with functional groups in biomolecules are indicated (W1 to W4). **(b)** Vector-normalize second-derivative of spectral windows associated with **(A)** lipids-W1 (3,000-2,800 cm^{-1}), **(B)** W2 assigned to protein absorptions (1,800-1,500 cm^{-1}), **(C)** W3-mixed regions (1,500-1,200 cm^{-1}), **(D)** W4 assigned to carbohydrates (1,200-900 cm^{-1}).

In addition, two bands sensitive to anomeric configuration assigned to α and β-glycosidic linkages in the region 900–800 cm^{-1} (904 and 842 cm^{-1} respectively) were observed. Specifically, a reduction in the level of β-1,4 glycosidic bonds resulted from the fungal activity, although it did not vary during the whole incubation period (Table III). In addition, absorbance FTIR spectra of uninoculated GP during 90 incubation days only showed some slight alterations in functional groups related to lignin-like aromatic polymers such as phenolic groups seen at 1241 cm^{-1} (data not shown). To better visualize the differences in the chemical modifications of GP by *Peniophora albobadia*, a PCA was applied (Fig. 4). The score-plot for PC2 and PC3 showed that the GP samples were distributed from left to right of PC2 axis according to the exposure time to the fungus. Interestingly, samples treated with the fungus for 90 days grouped separately from the rest (Fig. 4a). Bands corresponding to cellulose, hemicelluloses, and lignin proved to be spectral features that explained this discrimination, such as that analyzed in the loading plot (Fig 4b).

Different assays on the WSF of uninoculated and inoculated GP were performed. Only β-1,4 endoglucanase activity was found in WSF of inoculated GP, which did not vary during the whole incubation period (25.22 ± 6.10 $mU.ml^{-1}$, $p > 0.05$). In addition, the fungus modified several chemical parameters in the WSF, which presented a differential behavior (Table IV). Although some reduction in the free phenol content in WSF was observed in uninoculated GP after 90 incubation days, the fungus reduced the level of free phenols and TSRC in WSF during the whole incubation period. Compared to control GP, the E_4/E_6 ratio in WSF showed higher levels only in the 30-60 day period. However, similar E_4/E_6 ratios were found for both inoculated and uninoculated GP at 90 incubation days. Besides, ammonium and pH levels in WSF increased after 60 days in the presence of the fungus.

DISCUSSION

GP is a solid waste from the winemaking process that has a complex chemical composition. It contains grape peel and seeds as well as remaining pulp, in which cellulose, hemicelluloses, lignin, and pectin as well as soluble compounds such as phenolics and sugars are present in different proportions (Guerra-Rivas et al. 2017). The chemical composition of grapes and their residues produced in the wine industry varies among different *Vitis* species and the cultivar type, and other factors including the soil where the plant grows, the cultivation conditions, the stage of maturity, and the winemaking process (Guerra-Rivas et al. 2017, Schaffer et al. 2016). Compared to *V. vinifera* wine, the sensory characteristics from wine made from *V. labrusca* grapes have been associated with the content of several compounds such as flavonoids, organic acids and volatile phenols, which influence the color, flavor, bitterness, and astringency of wine (Ivanova-Petropulos et al. 2015, Arcanjo et al. 2017). Since Rizzon et al. (2000) reported that the skin of the Isabella grape yields a higher concentration of organic acids than that of *V. vinifera* cultivars, possibly due to the release of organic acids during maceration, the high concentration of these acids in GP using *V. labrusca* might in turn condition the selection of biological agents to reduce the wastes generated during the winemaking at Cooperativa del vino de la Costa (Argentina). Among the organisms involved in the revalorization of organic wastes, fungi, specifically WRF, have been reported to play a greater role than bacteria due to their ability to degrade severely recalcitrant plant polymers and several toxic simple compounds,

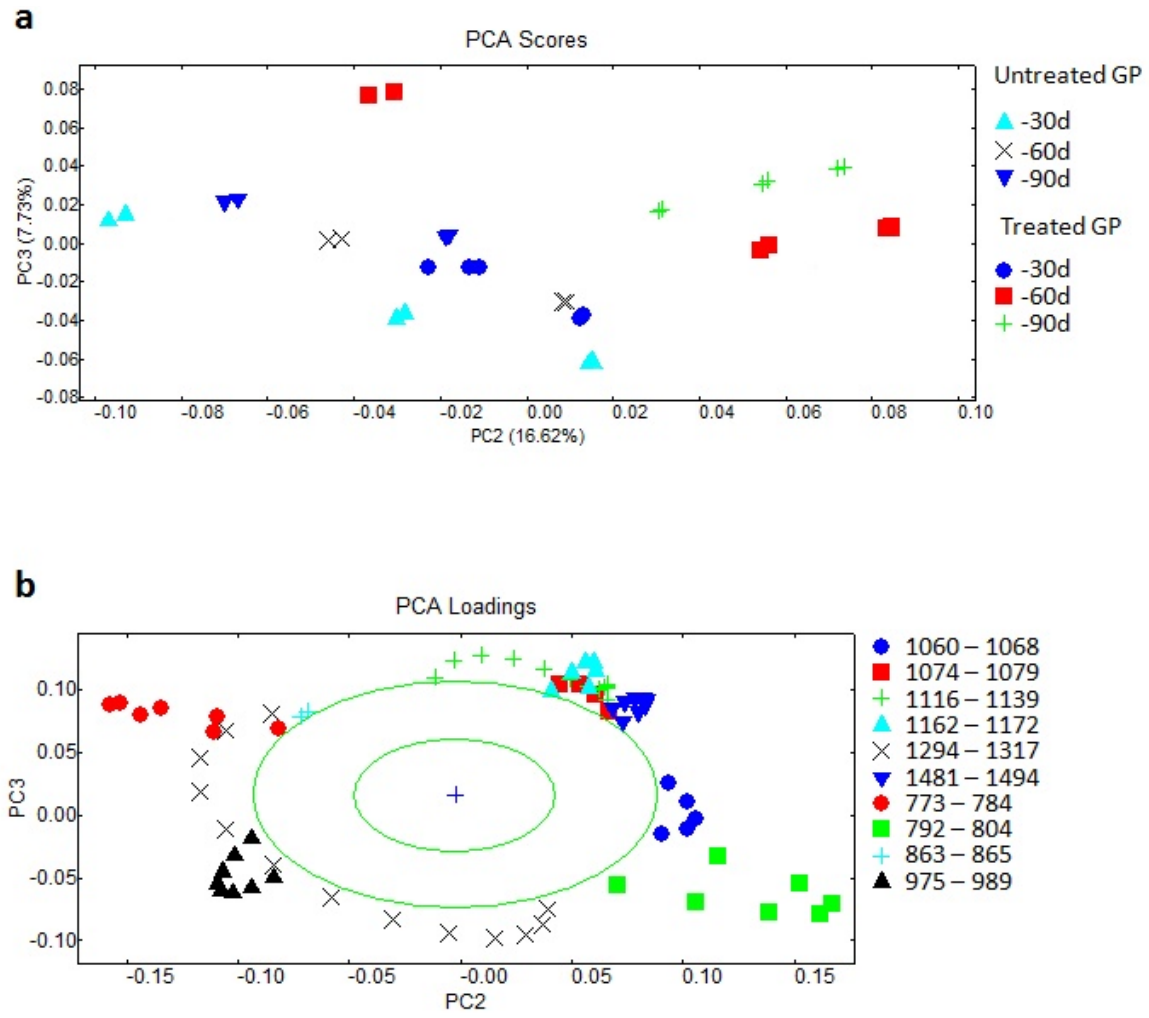

Figure 4. PCA of FT-IR average spectra of GP untreated and treated with *P. albobadia* LPSC 285 at several incubation times (30, 60, and 90 days). PCA was carried out with vector-normalized first-derivative spectra of three independent replicates of each treatment in the spectral range of W1 (3000-2800 cm^{-1}), W3 (1500-1250 cm^{-1}), and W4 (1200-750 cm^{-1}). (a) PCA score plot. (b) PCA loading.

such as phenolic compounds, as well as their ability to recycle nitrogen from endogenous sources more efficiently than bacteria (Hodge et al. 2000). Five WRF were used for treating GP from *V. labrusca* under SSF axenic conditions. These WRF are known as efficient lignin degraders of the natural woody substrates from where they are isolated, but there are no data about their degrading ability on wastes from *Vitis* spp. Although these fungi can degrade *in-vitro* GP and its components such as free phenolic compounds, they reduced GP mass differently among the tested fungi. This difference can be due, at least in part, to the ability of these fungi to detoxify inhibiting compounds and/or to synthetize different systems of ligno-cellulolytic enzymes. However, here we only selected *P. albobadia* LPSC 285 to monitor its potential in the transformation of GP under SSF conditions since it caused the highest substrate mass loss and removal of free phenols after 30 days of growth. Previous reports have shown

Table III. Semi-quantitative determination of β-glycosidic bonds in *Peniophora albobadia*-treated and untreated GP at different incubation times evaluated by FT-IR spectroscopy.

		$\alpha = A_{904}/A_{1262}$	
Culture time (days)		UT	T
	30	0.34 ± 0.05a	0.28 ± 0.02b
	60	0.32 ± 0.01a	0.26 ± 0.02b
	90	0.33 ± 0.02a	0.25 ± 0.05b

(α) Relative absorbance ratio for band area at 904 cm^{-1} (β-glucosidic bonds) and the area at 1262 cm^{-1} (C-O stretching of guaiacyl unit of lignin). Data followed by the same letter are not significantly different (Tukey's, $p < 0.05$). (UT) Untreated; (T) treated.

Table IV. Time course of transformation of GP untreated (UT) and treated (T) with Peniophora albobadia LPSC 285: physico-chemical parameters measured on the WSF.

Physico-chemical parameters	30 days[d]		60 days		90 days	
	UT	T	UT	T	UT	T
pH	3.67[a] ± 0.01 c	3.84 ± 0.02 b	3.62 ± 0.03 c	4.18 ± 0.07 a	3.63 ± 0.06 c	4.10 ± 0.02 a
Phenolics (mg.100 ml^{-1})	37.24 ± 3.72 a	5.97 ± 0.82 d	23.59 ± 1.78 b	5.50 ± 0.61 d	15.05 ± 3.95 c	5.35 ± 1.05 d
NH_4^+-N (mg.100 ml^{-1})	14.34 ± 2.68 b	12.66 ± 2.67 b	25.5 ± 3.32 b	105.80 ± 18.20 a	19.40 ± 3.50 b	108.59 ± 21.47 a
TRSC[b] (mM)	4.64 ± 0.32 a	1.66 ± 0.16 b	4.05 ± 1.00 a	0.77 ± 0.21 b	3.31 ± 0.34 a	0.56 ± 0.12 b
E_4/E_6[c]	5.83 ± 0.22 c	12.33 ± 0.79 a	4.51 ± 0.66 c	12.89 ± 0.59 a	9.24 ± 0.57 b	8.43 ± 0.22 b

[a]Mean and standard deviation of three biological replicates. Data followed by the same letter are not significantly different (Tukey's test, $p < 0.05$). [b](TRSC) Total soluble reducing carbohydrates. [c](E4/E6) Polydispersion index calculated as Abs. 465 nm * Abs. 665 nm^{-1} ratio. [d](UT) Untreated; (T) treated.

the outstanding ability of *P. albobadia* to attack wood and several of its components (Saparrat et al. 2000, 2002, Speranza et al. 2009). In the first period of incubation time (30 days), *P. albobadia* LPSC 285 showed the highest reduction in free phenols and TSRC available in WSF from GP. This high reduction was possibly due to the need to detoxify available phenols and use simple sugars as a source of energy and C, without modifying the ammonium nitrogen level in WSF because the fungus re-cycles the N upon mycelial autolysis. Similar results were found by analyzing cultures of other WRF grown on wheat straw, alpeorujo, and grape stalk (Dorado et al. 1999, Arienzo et al. 2003, Levin et al. 2012). Then, at 60 days, fungal-treated GP showed a higher amount of ammonium in WSF than the untreated one, which shows the fungus ability to mineralize organic N from GP. Nitrogen in wine pomace is mainly incorporated into proteins (6-15%, dry

matter), which are apparently covalently linked to polysaccharides in skin and seed (Toaldo et al. 2013, García-Lomillo & Gonzalez-San José 2017). In addition, the increase in ammonium content in WSF during the fungal transformation might be associated with the increase in pH in the WSF from GP. Therefore, the chemical mechanism behind the little rise in pH by GP treated by the fungus might be, at least in part, related to nitrogen metabolism.

FTIR spectroscopy has been used to characterize the chemistry of wood and determine the lignin content in pulp, paper and wood (Popescu et al. 2009). It has also been applied to analyze the chemical changes occurring during wood weathering, decay, and chemical treatments or natural ageing (Popescu et al. 2006). Several studies have used different spectroscopic technologies to analyze the fungal decay of wood (Pandey & Pitman 2003, Popescu at al. 2010). Since the interference of water vapor bands did not allow us to perform the FTIR analysis of protein bands in GP, future studies are needed to confirm if *P. albobadia* LPSC 285 has the ability to degrade GP proteins and to determine whether the proteins are the main source for the alkalinization processes mediated by this fungus. Nevertheless, this work represents the first detailed FT-IR spectroscopic analysis to monitor the chemical composition changes occurring during the treatment of GP by a white-rot fungus such as *P. albobadia* LPSC 285 isolate. Through this type of analysis, we detected a lower relative concentration of β-glycosidic bond in fungal treated GP, compared to the uninoculated one. This, together with the detection of β-1,4 endoglucanase activity in the WSF, confirmed that the fungus has the ability to degrade holocellulose. However, the activity of this enzyme and the level of the β-glycosidic bond in treated GP did not vary over time, which is compatible with the main action of the fungus degrading GP and its components on the first 30 days of incubation. Botella et al. (2007) also used GP as growth substrate to produce hydrolytic enzymes by *Aspergillus awamori*. Among extracellular oxidative enzymes, laccases and peroxidases are those mainly related to transformation of phenolics (Saparrat et al. 2010b). However, no activity of oxidative enzymes was found in WSF. These enzymes in *P. albobadia* LPSC 285, which were previously reported when the fungus was cultured on agar and liquid medium (Saparrat et al. 2000), might be immobilized in GP matrix and/or in fungal walls when cultured on SSF. Furthermore, these enzymes can be inhibited by the phenols available in the WSF from GP since previous data reported that lignocellulose substrates, including some from winemaking, and/or their phenols can inactivate extracellular laccases and MnPs (Ruiz et al. 2002, Skoronski et al. 2014). Since phenol reduction in WSF was observed for *P. albobadia* LPSC 285 and other fungi tested, polymerization of GP phenols mediated by the activity of extracellular laccases and/or peroxidases cannot be discarded (Saparrat et al. 2010b). Furthermore, the action of other concomitant fungal mechanisms could also be involved in phenol content reduction, as previously suggested (de la Rubia et al. 2008). Since a higher reduction was found at 30 days, the phenol removal in WSF from GP could also involve non-enzymatic mechanisms. Mycelial adsorption has been involved in detoxification and/or decolorization reactions of chromophoric components, including those available in other phenol-rich organic residues such as those from fungal transformation of alpeorujo (Saparrat & Hammer 2006, de la Rubia et al. 2008). Therefore, the decrease in sugars and free phenol content in the GP by *P. albobadia* LPSC 285 might be the results of a concerted action of both endoglucanase and

oxidative non-enzymatic events. In addition, WSF can be used as a factor that indirectly reflects alteration of the insoluble lignocellulose macromolecular fractions (Dorado et al. 1999, Mallerman et al. 2018). Specifically, the E_4/E_6 ratio, measured by UV-VIS spectroscopy, has been used as an indicator of organic matter quality in WSF (Dorado et al. 1999, Saparrat et al. 2008). This ratio is considered to be inversely related to the degree of condensation and aromaticity of the chromophore substances and to their degree of humification/transformation (Stevenson 1994, Senesi et al. 2003). We found a higher E_4/E_6 ratio in WSF from fungal-treated GP after 30 and 60 days, which reflects a low degree of aromatization and presence of a relatively large proportion of aliphatic structures in organic compounds in WSF (Stevenson 1994). This suggests a dominance of soluble aliphatic chromophores, which are easily decomposable, as opposed to WSF from untreated GP, which is a source of molecules with a high degree of aromaticity and condensation that are harder to decompose. However, an opposite pattern was found after 90 days of incubation, possibly due to the C-demand of the fungus, which first used easily assimilable compounds and then led to the accumulation of products derived from lignin depolymerisation in WSF. Such events are accompanied by a high polydispersion, which implies a predominance of soluble compounds of low molecular weight that could be consumed by fungus and/or repolymerized during the conversion process. A similar pattern was observed during the transformation of rice straw in soil by *Phanerochaete chrysosporium* and *Streptomyces badius* (Huang et al. 2008), though the monitoring time was higher than that in our study.

CONCLUSIONS

We here showed the potential of *P. albobadia* LPSC # 285 for treating GP from *V. labrusca*, the main solid waste generated from winemaking at "La Cooperativa de la Costa" in Argentina. This fungus transformed this residue, reducing its mass and soluble phenolic compounds. It metabolized simple sugar and soluble phenols and degraded polysaccharides available in this recalcitrant residue. Future studies are required to evaluate whether this fungus is also competitive on GP from other species of *Vitis,* such as *V. vinifera,* even under non-sterile conditions for large-scale applications, such as wineries.

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