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Sugar−Glycerol Cofermentations by *Lactobacillus hilgardii* Isolated from Wine

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Glycerol catabolism was studied in *Lactobacillus hilgardii* X_1B from wine, growing on glycerol and limiting glucose or fructose concentrations in anaerobiosis and microaerophilia. Glycerol consumption occurred simultaneously with sugar use, and it was higher with fructose as a cofermenting sugar in microaerophilia. Enzymatic activities of the glycerol kinase and glycerol dehydratase pathways were detected in both incubation conditions. In anaerobiosis, the main products were lactate, acetate, ethanol, and the intermediary product of the glycerol dehydratase pathway, 3-hydroxypropionaldehyde. However, in microaerophilia, 1,3-propanediol was also detected. In anaerobic glucose + glycerol and fructose + glycerol cultures as in microaerophilic glucose + glycerol cultures, glycerol was degraded mainly through the reductive pathway. However, when *L. hilgardii* X_1B was grown on fructose + glycerol cultures in microaerophilia, glycerol dissimilation occurred mainly via the glycerol kinase way. According to these results, *L. hilgardii* X_1B can degrade glycerol by producing 3-hydroxypropionaldehyde and acetic acid, both undesirable products for wine sensorial quality.

KEYWORDS: Glycerol catabolism; *Lactobacillus hilgardii*; wine

INTRODUCTION

Glycerol is quantitatively the main product of yeast fermentation during winemaking in addition to ethanol and carbon dioxide (*1*). It is a nonvolatile compound that lacks aromatic properties but significantly contributes to the wine quality by providing sweetness and fullness (*2*).

Lactic acid bacteria (LAB) play a key role in the malolactic fermentation of wines (*3*). After alcoholic fermentation, when sugars are exhausted, glycerol can be used by LAB to maintain its viability and, dependent upon the way that they are used for degrading it, they can be responsible of modifications in wine quality (*4*). Consequently, in fermented beverages, such as wines and ciders, bitterness is an alteration characterized by an unpleasant bitter taste, which is associated with the presence of acrolein. The combination of acrolein with polyphenols leads to the development of bitter compounds. Thus, LAB belonging to the genus *Lactobacillus* have been described as responsible for bitterness when glycerol is metabolized through the reductive pathway of glycerol dehydratase (GDA) (*5*, *6*). Glycerol is dehydrated into 3-hydroxypropionaldehyde (3-HPA) by a coenzyme B_{12}-dependent GDA. 3-HPA can be transformed into acrolein by chemical dehydratation under acidic and/or heat conditions (*7*). The 3-HPA is subsequently reduced to 1,3-propanediol (1,3-PD) by a NADH-dependent 1,3-propanediol dehydrogenase (1,3-PDH) (*5*, *8*) or can be oxidized to 3-hydroxypropionic acid (*7*) (**Figure 1**).

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The GDA pathway was demonstrated in heterofermentative lactobacilli, such as *L. brevis* and *L. buchnerii* (*8*), *L. collinoides* (*6*, *9*, *10*), some *L. hilgardii* strains (*11*), *L. coryniformes* (*12*), and *L. reuteri* (*13*, *14*). These last two species can synthesize 3-HPA, which excreted into the medium forms, together with the HA hydrate and HA dimer, a dynamic multicomponent system (HPA system) called reuterin (*15*). Reuterin is active toward Gram-positive and Gram-negative bacteria, yeasts, fungi, protozoa, and viruses (*16*). As a consequence, the use of *L. reuteri* as probiotic has been proposed (*12*, *14*).

Other metabolic pathways for glycerol catabolism by LAB from wine have been described. For instance, the glycerol kinase (GK) pathway that involves GK, which phosphorylates glycerol to glycerol 3-phosphate (G3P), and a NAD-dependent or NAD-independent G3P dehydrogenase (G3PDH), which oxidizes G3P to dihydroxyacetone phosphate (DHA-P) (**Figure 1**).

The glycerol dehydrogenase (GDH) pathway includes GDH that transform glycerol into dihydroxyacetone (DHA). The DHA is consequently phosphorylated to DHA-P by a DHA-kinase (DHAK). From DHA-P, the cells are able to conduct glycerol catabolism through the Embdem−Meyerhof−Parnas pathway (**Figure 1**). Thus, among the homofermentative LAB from wine, the GK pathway has been reported in *L. casei* Cog 812 (*17*) and microaerophilic and aerobic *Pediococcus pentosaceus* N_5p growth. This last strain is also able to degrade glycerol by the GDH way under microaerophilic conditions (*18*−*20*). If these pathways are involved in glycerol degradation, it could lead to the

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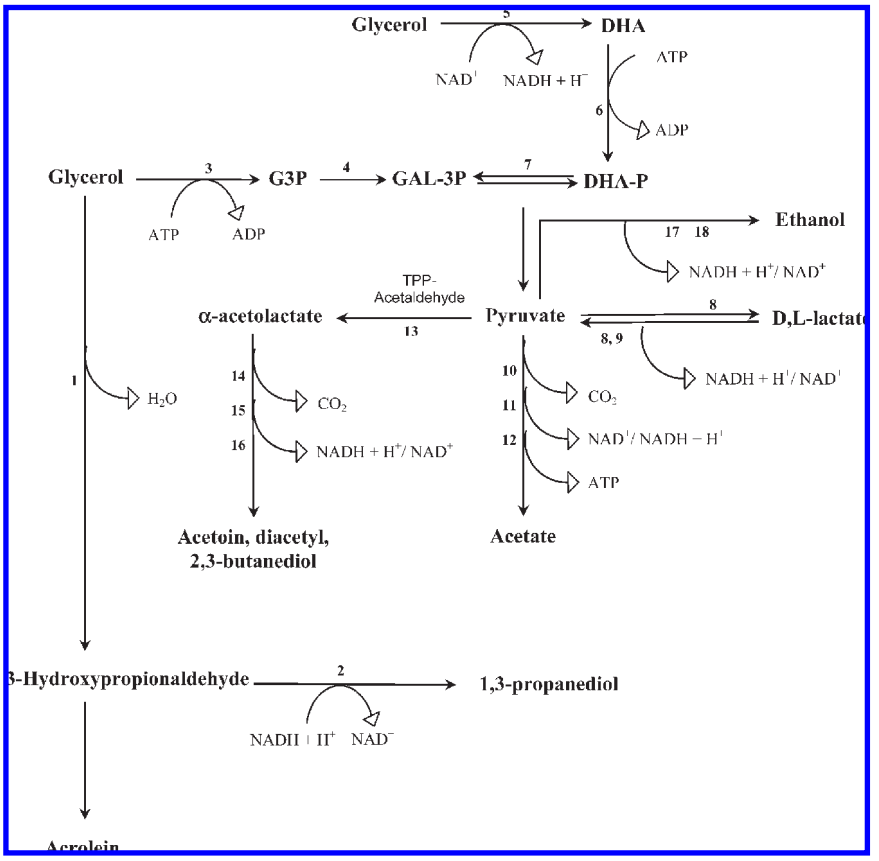

Figure 1. Metabolic pathways involved in glycerol catabolism in LAB: (1) glycerol dehydratase, (2) 1,3-propanediol dehydrogenase, (3) glycerol kinase, (4) NAD-dependent or NAD-independent glycerol 3-P dehydrogenase, (5) glycerol dehydrogenase, (6) dihydroxyacetone kinase, (7) triose phosphate isomerase, (8) NAD-dependent lactate dehydrogenases, (9) NAD-independent lactate dehydrogenase, (10) pyruvate dehydrogenase, (11) phosphotransacetilase, (12) acetate kinase, (13) α-acetolactate synthase, (14) α-acetolactate decarboxylase, (15) diacetyl reductase, (16) acetoin reductase, (17) acetaldehyde dehydrogenase, and (18) alcohol dehydrogenase.

production of compounds, such as diacetyl, acetoin, and acetic acid, which can alter the flavor of wine.

There are no references about glycerol catabolism in *L. hilgardii* strains from wine. Taking into account the fact that this heterofermentative species could be isolated from some spoiled wine (*21*), the purpose of this work was to study the catabolism of glycerol in the selected strain *L. hilgardii* X_1B. We investigated the enzymatic activities involved in glycerol degradation as well as the influence of substrates and atmospheres of incubation in the formation of products related to wine-quality alterations.

MATERIALS AND METHODS

Bacterial Strain and Culture Conditions. *L. hilgardii* X_1B was isolated from an Argentinean wine (*21*). It was maintained by a subculture in MRS medium (*22*) at pH 6.5 and 30 °C.

To study glycerol catabolism, the strain was grown in MRS medium at pH 6.5, modified by suppressing acetate and citrate with the addition of an appropriate carbon source: 5.5 mM glucose or fructose and 43.2 mM glycerol.

Microaerophilic growth was conducted in unshaken capped tubes or flasks two-thirds full (750 mL culture medium), incubated for 48 h at 30 °C.

Anaerobic cultures were incubated, unshaken, in completely filled tubes (1000 mL culture medium) for 96 h at 30 °C in a CO_2 incubator under air supplemented with 5% CO_2.

For each growth condition, several flasks were inoculated. This technique was preferred to the alternative single large culture to avoid aeration during sampling, which would disturb the experimental conditions.

Growth was determined by measuring optical density at 660 nm.

Analytical Methods. After bacterial growth, the concentrations of substrates and end products were determined in cell-free supernatants of cultures. Glucose, fructose, and mannitol were determined by high-pressure liquid chromatography. Samples (20 μL) were analyzed using a Knauer system chromatograph fitted with an IR detector K-2301 (Knauer) and an oven ZC90 (Zeltec). The compounds were separated on a Hamilton HC-75 column at 70 °C and eluted with double-distilled water, at a flow rate of 1.2 mL min^{-1}. The analysis was internally calibrated using standard solutions prepared in double-distilled water.

1,3-propanediol, diacetyl, acetoin, and 2,3-butanediol were determined by gas chromatography (GC). Samples (2 μL) were analyzed using a HP 5890 gas chromatograph fitted with a flame ionization detector. The compounds were separated on a HP-Innowax (cross-linked polyethylene glycol) column: 30 m × 0.53 mm inner diameter, with a film thickness of 1.0 μm. Nitrogen was used as a carrier gas at a flow of 2.6 mL min^{-1}. The GC temperature program was as follow: 40 °C for 2.5 min and then increased at 5 °C min^{-1} to 228 °C for 20 min. The analysis was externally calibrated using standard solutions prepared in ethanol. The identity of compounds in samples was verified using GC−mass spectroscopy.

Glycerol, D- and L-lactate, ethanol, and acetate were quantified using kits supplied by Boehringer-Mannheim, Inc. (Germany).

3-HPA determination was based on the colorimetric method of Circle et al. (*23*).

Preparation of Cell-Free Extracts. Cells from 1000 mL cultures of *L. hilgardii* X_1B, at the end of logarithmic phase of growth, were harvested by centrifuging at 7000*g* for 15 min at 4 °C. The pellet were washed twice with 50 mM Tris-HCl buffer at pH 7.5, suspended at 30% (w/v) in the same buffer, and

disrupted in a French press. The cell-free extracts were obtained by centrifuging at 30000*g* for 30 min at 4 °C.

Enzymatic Activities Assays. To assay GK activity, the reaction mixture contained 50 mM Tris-HCl buffer at pH 7.5, 3.2 mM $MgCl_2$, 1 mM ATP, 100 mM glycerol, and 300 μL of cell-free extract. After 5 min at 25 °C, the reaction was started with 1.47 mM phosphoenolpyruvate, 0.27 mM NADH, and 2×10^3 units L^{-1} of both pyruvate kinase and L-lactate dehydrogenase. NADH oxidation was followed at 340 nm at 25 °C (*19*).

For the determination of GDH activity, the reaction mixture contained 50 mM Tris-HCl buffer at pH 7.5 and 9.0 (*24*), 30 mg L^{-1} NAD, 1.8 mM $MgCl_2$, 1.5 mM glycerol, and 300 μL of cell-free extract. NAD reduction was followed at 340 nm at 25 °C. NAD- and FAD-dependent glycerol 3-phosphate dehydrogenase activities were determined in the same reaction mixture but replacing glycerol with G3P (*19*).

The reaction mixture for DHAK activity contained 50 mM potassium phosphate buffer at pH 7.0, 1 mM $MgCl_2$, 1 mM ATP, 1 mM DHA, 0.133 mM NADH, 2 μg mL^{-1} α-glycerophosphate dehydrogenase, 5 mM glycerol, and 10 mM α−α-dipyridyl, and 300 μL of cell-free extract. NADH oxidation was followed at 340 nm at 25 °C (*24*). GK has a much higher apparent affinity for glycerol than DHAK. Fortunately, DHAK does not act on glycerol or is inhibited by it, even at 100 mM. Therefore, the presence of 1 mM glycerol almost totally prevents the action of any GK on DHA without affecting the activity of DHAK. GDH also interferes with the assay by catalyzing the reduction of DHA at the expense of NADH. This reaction can be strongly inhibited by α−α-dipyridyl at 10 mM, under which condition there was no effect on DHAK (*25*).

GDA activity was determined by the 3-methyl-2-benzothiazolinone−hydrazone method described by Toraya et al. (*26*).

1,3-PDH activity was measured at 25 °C in 100 mM Tris-HCl buffer at pH 9.0 containing 155 mM 1,3- propanediol, 1 mM NAD, and 200 μL of cell-free extract, following NAD reduction at 340 nm (*24*, *27*).

One unit of enzyme activity is the amount of enzyme that catalyzes the formation of 1 μmol of reaction product per minute under the specific conditions of the particular assay used.

The protein concentration in crude extracts was determined by Bradford's method to allow for the calculation of specific activities.

Statistical Analysis. Experiments were carried out in triplicate. One-way analysis of variance was performed separately in anaerobic and microaerophilic conditions. Significant differences among treatments were computed using Tukey's test at a 0.05 level (Minitab Student R12) (*28*).

RESULTS

Growth Conditions. The growth of *L. hilgardii* X_1B in anaerobic and microaerophilic conditions, in the media containing limiting glucose or fructose and glycerol as main carbon sources, is shown in **Figures 2** and **3**. Even when good growth of *L. hilgardii* X_1B was observed in both incubation conditions with glucose or fructose as carbon sources, this growth was enhanced in microaerophilia with the highest growth rate value.

The strain was unable to grow on glycerol as its sole carbon source, independent of the incubation condition. Consequently, the addition of glucose or fructose to glycerol medium was necessary for *L. hilgardii* growth. In these conditions, glycerol was cofermented with sugars and a diminution in the final biomass and growth rates in both media (glucose + glycerol and fructose + glycerol) and incubation conditions was detected.

Substrates Consumption and End-Products Formation by *L. hilgardii* X_1B. Substrates and end products were quantified at the end of *L. hilgardii* growth on sugars or sugar + glycerol media in both anaerobic (**Table 1**) and microaerophilic (**Table 2**) conditions.

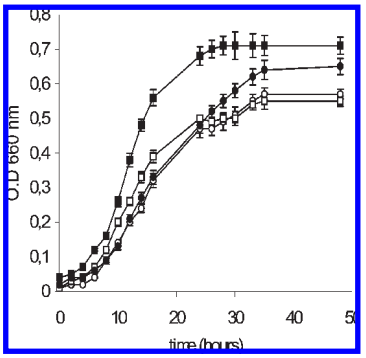

Figure 2. Growth of *L. hilgardii* X_1B in microaerophilia. Modified MRS medium containing: glucose (●), fructose (■), glucose + glycerol (○), and fructose + glycerol (□).

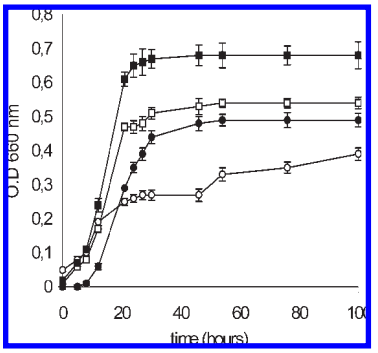

Figure 3. Growth of *L. hilgardii* X_1B in anaerobiosis. Modified MRS medium containing: glucose (●), fructose (■), glucose + glycerol (○), and fructose + glycerol (□).

In the media with limiting glucose and fructose concentrations, sugars were completely consumed by *L. hilgardii* X_1B by producing D- and L-lactate, acetate, ethanol, CO_2, and a little amount of mannitol in the fructose medium. In both incubation conditions, the products were the same in either glucose or fructose media. In anaerobiosis, L-lactate was the main isomer, whereas in microaerophilia, D-lactate was predominant but less total lactate and more acetate and ethanol were produced.

When glycerol was added to the sugars media in anaerobiosis, glycerol consumption was higher with fructose (17.45%) than glucose (14.6%). The end products detected were lactate, acetate, ethanol, and the intermediary product of the GDA pathway: 3-HPA. In these conditions, there was an enhancement in lactate, acetate, and ethanol concentrations with respect to the sugars media. A total of 57.7 and 64% of glycerol were recovered as 3-HPA in glucose + glycerol and fructose + glycerol cultures, respectively.

In microaerophilic conditions, glycerol consumption on glucose + glycerol cultures (12.8%) was higher than that observed in anaerobiosis and 55% of glycerol was recovered as the GDA pathway products: 3-HPA and 1,3-propanediol (1,3-PD). On fructose + glycerol cultures, glycerol use (23.2%) was higher than in anaerobic conditions and only 34% of glycerol was recovered as 3-HPA and 1,3-PD.

Mannitol was not detected on fructose + glycerol cultures in any incubation conditions.

Under our experimental conditions, no diacetyl, acetoin, and 2,3-butanediol were found.

To determine the carbon recovery, the theoretical calculation of CO_2 produced was carried out and, in all conditions, recovery values between 98 and 99.2% indicated that all products formed have been detected.

Table 1. Glycerol and Sugars Consumption in Anaerobic Cultures of *L. hilgardii* X_1B: Substrates and Products at the End of Growth[a]

	substrate consumption (mM)			end products (mM)							
carbon source[b]	Glu	Fru	Gly	D-lactate	L-lactate	acetate	ethanol	mannitol	3-HPA[c]	CO_2[d]	% CR[e]
glucose	5.50 ± 0.26			2.47 ± 0.12 a	3.20 ± 0.19 a	2.84 ± 0.12 a	2.40 ± 0.18 a	0.0 a	0.0 a	5.24 ± 0.20 a	98.5
fructose		5.50 ± 0.30		2.28 ± 0.10 a	3.30 ± 0.20 a	2.72 ± 0.19 a	1.98 ± 0.08 b	0.34 ± 0.03 b	0.0 a	4.70 ± 0.32 a	99.2
Glu + Gly	5.50 ± 0.28		6.34 ± 0.29	3.55 ± 0.21 b	3.10 ± 0.10 a	3.57 ± 0.33 b	3.26 ± 0.18 c	0.0 a	3.65 ± 0.23 b	6.83 ± 0.36 b	98.8
Fru + Gly		5.50 ± 0.21	7.54 ± 0.32	3.11 ± 0.15 c	2.63 ± 0.12 b	4.23 ± 0.29 b	3.50 ± 0.16 c	0.0 a	4.86 ± 0.33 c	7.73 ± 0.45 b	98.9

[a] Data are expressed as means ± standard deviations. Treatment means followed by the same letter do not differ significantly at the 0.05 level according to Tukey's test. [b] Carbon source: Glu, glucose (5.50 mM); Fru, fructose (5.50 mM); Gly, glycerol (43.20 mM). Products: [c] 3-HPA, hydroxypropionaldehyde. [d] The CO_2 concentration was calculated considering its generation by pyruvate dehydrogenase: 1 mol of CO_2/mol of acetate, 1 mol of CO_2/mol of ethanol, and 1 mol/mol of hexose catabolized. [e] % CR = carbon recovery as a percentage.

Table 2. Glycerol and Sugars Consumption in Microaerophilic Cultures of *L. hilgardii* X_1B: Substrates and Products at the End of Growth[a]

	substrate consumption (mM)			end products (mM)								
carbon source[b]	Glu	Fru	Gly	D-lactate	L-lactate	acetate	ethanol	mannitol	3-HPA[c]	1,3-PD[d]	CO_2[e]	% CR[f]
glucose	5.50 ± 0.18			2.84 ± 0.18 a	1.73 ± 0.10 a	3.24 ± 0.19 a	3.00 ± 0.16 a	0.0 a	0.0 a	0.0 a	6.24 ± 0.22 a,b	98.3
fructose		5.50 ± 0.27		2.93 ± 0.13 a	1.95 ± 0.14 a	2.93 ± 0.21 a	2.53 ± 0.11 b	0.25 ± 0.02 b	0.0 a	0.0 a	5.46 ± 0.31 a	98.5
Glu + Gly	5.50 ± 0.25		5.43 ± 0.33	4.12 ± 0.28 b	2.40 ± 0.13 b	4.08 ± 0.34 b	2.70 ± 0.14 a,b	0.0 a	1.08 ± 0.07 b	1.90 ± 0.13 b	6.78 ± 0.46 b	99.0
Fru + Gly		5.50 ± 0.29	9.81 ± 0.67	5.84 ± 0.27 c	4.20 ± 0.23 c	4.19 ± 0.29 b	2.80 ± 0.17 a,b	0.0 a	0.88 ± 0.06 c	2.51 ± 0.16 c	6.99 ± 0.59 b	98.0

[a] Data are expressed as means ± standard deviations. Treatment means followed by the same letter do not differ significantly at the 0.05 level according to Tukey's test. [b] Carbon source: Glu, glucose (5.50 mM); Fru, fructose (5.50 mM); Gly, glycerol (43.20 mM). Products: [c] 3-HPA, 3-hydroxypropionaldehyde; [d] 1,3-PD, 1,3-propanediol. [e] The CO_2 concentration was calculated considering its generation by pyruvate dehydrogenase: 1 mol of CO_2/mol of acetate, 1 mol of CO_2/mol of ethanol, and 1 mol/mol of hexose catabolized. [f] % CR = carbon recovery as a percentage.

Table 3. Enzymatic Activities Involved in Glycerol Catabolism by *L. hilgardii* X_1B[a]

	specific activities (units mg^{-1})				
culture conditions[b]	GK[c]	G3PDH[d]	GDA[e]	1,3-PD-DH[f]	GDH/DHAK[g]
Glu−Gly−Ana	6.28 ± 0.25 a	7.41 ± 0.27 a	5.63 ± 0.24 a	1.39 ± 0.05 a	ND[h]
Glu−Gly−Micro	22.14 ± 1.09 a	45.1 ± 1.42 a	7.87 ± 0.40 a	10.31 ± 0.45 a	ND
Fru−Gly−Ana	9.17 ± 0.43 b	10.5 ± 0.48 b	5.78 ± 0. 23 b	0.72 ± 0.03 b	ND
Fru−Gly−Micro	16.65 ± 0.99 b	33.7 ± 1.34 b	5.63 ± 0.32 b	7.90 ± 0.38 b	ND

[a] Data are expressed as means ± standard deviations. Treatment means followed by the same letter do not differ significantly at the 0.05 level according to Tukey's test. [b] Carbon sources concentration: glycerol (Gly), 43.20 mM; glucose (Glu), 5.50 mM; and fructose (Fru), 5.50 mM. *L. hilgardii* cultures were incubated in anaerobic (Ana) and microaerophilic (Micro) conditions. [c] GK = glycerol kinase. [d] G3PDH = NAD-dependent glycerol 3-phosphate dehydrogenase. [e] GDA = glycerol dehydratase. [f] 1,3-PDH = 1,3-propanediol-dehydrogenase. [g] GDH/DHAK = glycerol dehydrogenase/dihydroxyacetone kinase. [h] ND = no detected.

Enzymatic Activities. The enzymatic activities of GK, GDA, and GDH pathways were evaluated in cell-free extracts of *L. hilgardii* X_1B growing in anaerobic and microaerophilic conditions (**Table 3**). Levels of GDA activity were similar in both incubation conditions in fructose + glycerol cultures as well as in anaerobic glucose + glycerol media, but it was higher in glucose + glycerol cultures in microaerophilic conditions. 1,3-PDH activities were about 10-fold higher in microaerophilia than in anaerobiosis.

In reference to the GK pathway, GK and G3PDH activities were also higher in microaerophilic than in anaerobic conditions.

Under our experimental conditions, the enzymes of the GDH pathway were not detected in both incubation conditions.

DISCUSSION

L. hilgardii is a species found in some Argentinean wines in which the lactic microbiota is heterogeneous (*21*). Although *Oenococcus oeni* strains contribute to wine quality (*3*, *4*), other LAB species (for example, heterofermentative lactobacilli) would participate as spoilage microorganisms. This work provides information about the ability of *L. hilgardii* X_1B to degrade glycerol under anaerobic and microaerophilic conditions using culture media containing limiting glucose or fructose and glycerol. Sugars and glycerol concentrations were selected according to those usually found in Argentinean wines when the alcoholic fermentation has finished (*21*).

Several factors can influence the fate of a substrate by different alternative metabolic pathways. Among them, the carbon source and the electron acceptors as oxygen can be involved (*29*). According to our results, *L. hilgardii* X_1B was unable to grow in media containing glycerol as its sole carbon source in either anaerobiosis or microaerophilia. Glycerol use as its sole carbon source is possible when the two pathways of the "*dha* regulon" (GDA and GDH) are present, as reported in *Klebsiella* species (*25*, *30*), *Clostridium butyricum* and *C. acetobutyricum* (*31*), *Citrobacter freundii* (*32*), and *Enterobacter agglomerans* (*33*). Under our experimental conditions, the enzymes of the GDH pathway were absent in *L. hilgardii* X_1B cell-free extracts.

The ability of *L. hilgardii* to grow and degrade glycerol on sugar + glycerol media agrees with the sugar−glycerol cofermentation reported for other heterofermentative lactobacilli, including *L. reuteri* (*5*, *34*), *L. brevis* (*27*), *L. buchnerii* (*8*), and *L. collinoides* (*6*, *9*, *10*), in which the GDA pathway is efficient. Moreover, the GDA activity was detected in some *L. hilgardii* strains isolated from spoiled ciders (*11*). According to our results, both the GK and GDA pathways operate for glycerol catabolism in *L. hilgardii* X_1B. The strain was able to

produce lactate, acetate, ethanol, and CO_2 from sugars according to their heterofermentative pattern. In the presence of glycerol, the enhancement of these products could be related to the activities of the GK pathway that catabolizes glycerol into pyruvate, from which lactate, ethanol, and acetate are produced. Ethanol formation contributes to recycling NADH by the reaction catalyzed by ethanol dehydrogenase. Acetate production from pyruvate is assumed to occur via acetyl phosphate by acetate kinase, generating additional ATP.

The *L. hilgardii* strain was also able to synthesize the products of the GDA pathway: 3-HPA in anaerobiosis and 3-HPA and 1,3-PD in microaerophilia. In fact, the GDA pathway provides 3-HPA, which is an alternative hydrogen acceptor for recycling NADH produced during the catabolism of hexose via the 6-phosphogluconate pathway. 3-HPA is a toxic metabolite and needs to be reduced to 1,2- or 1,3-PD to detoxify the cell. Moreover, the absence of 1,3-PD in anaerobic sugars + glycerol cultures would be related to the very low levels of 1,3-PDH activity detected.

In microaerophilic conditions, the addition of glycerol produced an increase in both lactate isomers and acetate production but the ethanol concentration remained without significant variation. This is probably due to the formation of 1,3-PD that supplies NAD. As a consequence, no further ethanol formation is needed; thus, more pyruvate is available to form lactate and acetate.

In anaerobic sugars + glycerol cultures, an increase in L-lactate production was not observed. Even more, there was a decreasing production of L-lactate on fructose + glycerol media. This is probably due to the oxidation of L-lactate produced from glycerol to pyruvate by NAD-independent lactate dehydrogenases, as reported for other LAB (*8*, *35*). The absence of mannitol in fructose + glycerol cultures in both incubation conditions suggests that the NADH reoxidation occurs in anaerobiosis via ethanol production, while in microaerophilia, the NADH reoxidation occurs through 1,3-PD synthesis.

Similar to other heterofermentative lactobacilli isolated from fermented beverages that convert glycerol in 3-HPA, a precursor of acrolein (*6*), which is an undesirable compound for human health (*11*), *L. hilgardii* X_1B produces 3-HPA, suggesting that this strain can be involved in the production of the bitter taste in wine. The glycerol-degrading strains are undesirable not only because they produce bitterness but also because they produce high-volatile acidity.

This paper reports for the first time the catabolism of glycerol in *L. hilgardii*, a heterofermentative lactobacilli isolated from wine, in which operates both the GK and GDA pathways. It seems that the ways to degrade glycerol in *Lactobacillus* are variable. According to the species, it may depend upon both the presence or absence of genes encoding specific enzymes and the conditions of their expression.

The study includes the detection of the enzymatic activities of the GK pathway, its relation to the GDA pathway, and the end products of both sugars and glycerol catabolism. The end products detected are in agreement with the enzymatic activity levels and growth parameters. It is probable that the expression of the enzyme is regulated to the oxygen availability and intracellular redox balance. This could be explained by the better expression of the 1,3-PDH activity and the GK pathway enzymes in the presence of oxygen, as reported for the GK way in *Pediococcus pentosaceus* from wine (*20*).

These results represent the basis to study the catabolism of glycerol by *L. hilgardii* X_1B in different winemaking conditions, taking into consideration that this strain can degrade glycerol by producing 3-HPA and acetic acid, both undesirable products for the sensorial quality of wines.

Further studies should be performed with other *L. hilgardii* strains, to verify if the glycerol catabolism is a real problem in winemaking or if it is sporadic.

ABBREVIATIONS USED

LAB, lactic acid bacteria; GC, gas chromatography; GDA, glycerol dehydratase; 1,3-PDH, 1,3-propanediol dehydorgenase; GK, glycerol kinase; G3PDH, NAD-dependent/independent G3P dehydrogenase; GDH, glycerol dehydrogenase; DHAK, dihydroxyacetone kinase; 3-HPA, 3-hydroxypropionaldehyde; 1,3-PD, 1,3-propanediol; G3P, glycerol 3-phosphate; DHA, dihydroxyacetone; DHA-P, dihydroxyacetone phosphate.

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