Effect of Gallic Acid and Catechin on *Lactobacillus hilgardii* 5w Growth and Metabolism of Organic Compounds

María R. Alberto,[†] Marta E. Farías,[‡] and María C. Manca de Nadra*[,‡]

Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán and Centro de Referencia para Lactobacilos (CERELA), Chacabuco 145, 4000 Tucumán, Argentina

The effects of different concentrations of (+)-catechin and gallic acid on the growth and metabolism of *Lactobacillus hilgardii* in different media were evaluated. These phenolic compounds at concentrations normally present in wine not only stimulated the growth rate but also resulted in greater cell densities during the stationary phase of growth in both media. During the first hours of growth both phenolic compounds activated the rate of glucose and fructose utilization and only catechin increased the malic acid consumption rate. Gallic acid and catechin were consumed from the beginning of *L. hilgardii* growth. All cited effects were increased when the cells were precultivated in the presence of phenolic compounds, especially in the FT80 medium. As stimulating agents of *L. hilgardii* 5w growth, gallic acid and catechin could increase the risk of spoilage lactic acid bacteria in wine.

Keywords: *Lactobacillus hilgardii; gallic acid; catechin*

INTRODUCTION

Grapes and wine contain a large array of phenolic compounds. There are three classes: one is composed of the nonflavonoids, which are derivatives of cinnamic and benzoic acid; the second includes the flavonoids; and tannins comprise the third group. Flavonoids and other plant phenolics have been reported to have multiple biological effects such as antioxidant activity, anti-inflamatory action, inhibition of platelet aggregation, and antimicrobial activities (*1*). The concentrations of total phenol varied from 1800 to 4059 expressed as milligrams per liter of gallic acid equivalents (GAE), averaging 2567 mg L^{-1} GAE, for the red wines and from 165 to 331 mg L^{-1}, averaging 239 mg L^{-1} GAE, for the white wines (*2*). Most of the phenolic compounds in grapes are flavonoids (80%), the most significant being the catechins (Figure 1a). They are found at levels of approximately 190 mg L^{-1} in red wines and 35 mg L^{-1} in white wines. The major phenolic acid in grapes is gallic acid (Figure 1b). Red wine was found to contain an average of 95 mg L^{-1} and white wine ∼7 mg L^{-1}. Phenolic compounds are responsible for red wine color, astringency, and bitterness, in addition to contributing somewhat to the olfactory profile of the wine (*3*). Grape variety, processing practices, and storage, among other factors, affect the phenolic composition of grape juices and wines (*4*). Bacteria may use tannins and phenols. These compounds may affect their growth and metabolism (*5*). Phenol carboxylic acids can inhibit or stimulate the growth of lactic acid bacteria (*6*, *7*), and some of them can stimulate malolactic fermentation carried out by *Œnococcus oeni* (*8*, *9*). Several strains of lactic acid bacteria isolated from wine were tested for their activities to metabolize ferulic and *p*-coumaric acids (*10*).

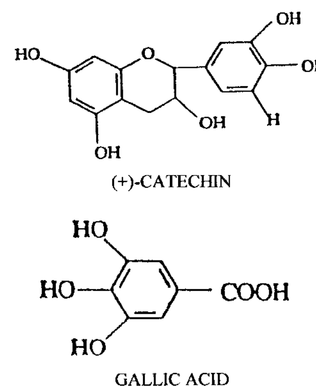

Figure 1. Chemical structures of gallic acid and (+)-catechin.

Barthelmebs et al. (*11*, *12*) reported that *Lactobacillus plantarum* displays substrate-inducible decarboxylase activities on *p*-coumaric, caffeic, and ferulic acids.

The present study was undertaken to determine the effect of the most common phenol carboxylic acid (gallic acid) and flavonoid (catechin) on the growth and metabolism in broth systems of *Lactobacillus hilgardii* 5w isolated from Argentinean wine (*13*). This bacterium has negative ecological effects, such as hydrogen peroxide and histamine production (*14*−*17*). The ultimate aim is to contribute to the selection of the mechanism to control microbial spoilage in wines.

MATERIALS AND METHODS

Microorganism. *Lactobacillus hilgardii* 5w was used for these experiments.

Culture Media. MRS (*18*) with added 15% tomato juice and FT80 (*19*) media were utilized. The pH was adjusted to 5.0 before sterilization. The first medium was selected for optimal conditions for *Lactobacillus* growth. FT80 was selected because of the possibility to compare with MRS the effect of phenolic compounds on the growth and also to study the utilization of malic acid and fructose.

* Author to whom correspondence should be addressed (e-mail mcmanca@unt.edu.ar; fax 54-381-4310465).

[†] Fellow of CONICET-Argentina.

[‡] Career Investigator of CONICET- Argentina.

10.1021/jf0101915 CCC: $20.00 © 2001 American Chemical Society
Published on Web 08/08/2001

Table 1. Effect of Different Wine Phenolic Compound Concentrations on *L. hilgardii* 5w Growth

MRS medium + phenolic compound	growth stimulation (%) at 12 h	growth stimulation (%) at 24 h
1000 mg L^{-1} GAE	10[a]	40
3000 mg L^{-1} GAE	12	20
6000 mg L^{-1} GAE	9	0

[a] Values are an average of three replicates with maximum standard deviation of <6%.

Growth Conditions. Tubes containing 30 mL of sterile medium were adjusted to various concentrations of wine total phenolic compounds (1000, 3000, and 6000 mg L^{-1} GAE) or with stock solutions 10 g L^{-1} in ethanol (25% v/v) of gallic acid (50, 100, and 200 mg L^{-1}) or catechin (100, 200, and 400 mg L^{-1}). The wine was concentrated by rotary evaporator before it was added to the basal media. Gallic acid was from Merck (Darmstadt, Germany), and (+)-catechin was from Sigma Chemical Co. (St. Louis, MO); purity of these compounds was 98%. Cells grown in basal medium with and without phenolic compounds were inoculated into the media FT80 and MRS containing the phenolic compounds. Inoculation (5% v/v) with bacteria was followed by incubation in darkness at 30 °C for 24 h in microaerophilic conditions. All cultures were carried out in triplicate. When gallic acid and catechin were included in the medium, they were added as filter-sterilized solutions to the autoclaved medium.

Growth Measurements. Bacterial growth was evaluated by direct measurement of optical density (OD 560 nm for MRS and OD 600 nm for FT80) and by plating 0.1 mL of adequate dilution of inoculated media on MRS and 20.0 g L^{-1} agar. Incubation time was 5 days at 30 °C in microaerophilic conditions.

Analytical Measurements. Colorimetric determination of total phenolics was based on the procedure of Singleton and Rossi (*20*). The sample was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent (Sigma). Two milliliters of saturated sodium carbonate (75 g L^{-1}) was added to the mixture and then shaken. The absorbance of the solution at 725 nm was measured after 2 h of incubation at 24 °C. Quantitation was based on the standard curve of gallic acid (Merck) or (+)-catechin (Sigma) prepared at the same time.

L-Malic acid was determined using an enzymatic kit from Boehringer (Mannhein, Germany), glucose was assayed by the glucose oxidase method from Wiener (Lab. Rosario, Argentina), and fructose was assayed according to the Roe method (*21*).

RESULTS

Effect of Total Wine Phenolic Compounds on Growth. Table 1 shows the growth of *L. hilgardii* 5w in MRS medium with added wine phenolic compounds. At 24 h of incubation at 30 °C, the higher growth stimulation was observed with 1000 mg L^{-1} GAE. The stimulation diminished proportionally to the increase of phenolic compounds, and with 6000 mg L^{-1} GAE the stimulatory effect disappeared.

Effect of Gallic Acid and Catechin on Bacterial Growth. The growth of *L. hilgardii* in MRS with increasing concentrations of gallic acid and catechin is shown in Figure 2. The exponential cell growth was faster in the gallic acid medium than in the control. The growth rate (μ_{max}) in basal medium was 0.36 h^{-1} and increased to 0.40, 0.46, and 0.41 h^{-1} in the media with 50, 100, and 200 mg L^{-1} gallic acid, respectively. In the stationary growth phase the population reached similar cell densities in media with 50 and 200 mg L^{-1} [$\sim 1.8 \times 10^{10}$ colony-forming units (cfu) mL^{-1}]. With gallic acid at a concentration normally present in red wine (100 mg L^{-1}) the final biomass increased to 2.0×10^{10} cfu mL^{-1}. Higher gallic acid concentrations showed an inhibitory effect on *L. hilgardii* 5w growth (10% and 26% for 1000 and 3000 mg L^{-1}, respectively).

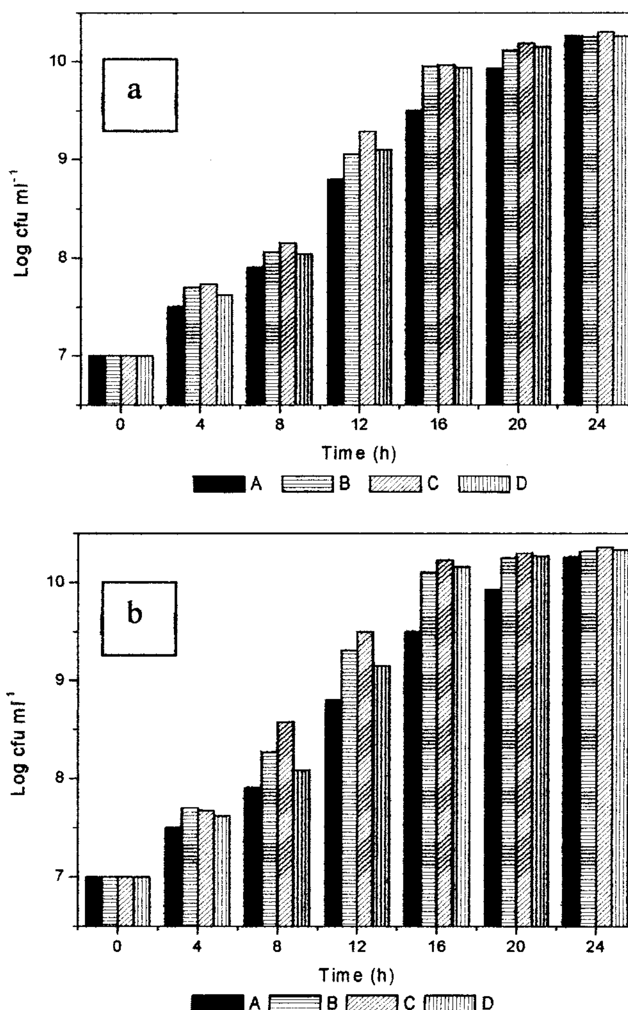

Figure 2. Influence of (a) gallic acid and (b) catechin at different concentrations (mg L^{-1}) on the growth of *L. hilgardii* 5w in MRS media: (A) control; (B) 50, (C) 100, and (D) 200 (for catechin 100, 200, and 400, respectively). Values are an average of three replicates with maximum standard deviation of <5%.

When catechin was included in the media, there was a growth rate stimulating effect, which increased up to 200 mg L^{-1}. The μ_{max} values were 0.36, 0.46, 0.52, and 0.44 h^{-1} for 0, 100, 200, and 400 mg L^{-1} catechin. In all cases, catechin increased the cell densities during the stationary growth phase, 200 mg L^{-1} being the more effective concentration (2.3×10^{10} cfu mL^{-1}). Populations of 2.1×10^{10} and 2.2×10^{10} cfu mL^{-1} were achieved in the presence of 100 and 400 mg L^{-1} catechin, respectively.

Phenolic Compounds Consumption. Gallic acid and catechin consumption by *L. hilgardii* at 24 h of incubation in MRS medium was directly proportional to the concentration of the phenolic compound added to the medium (Table 2). The phenolic acid consumption was 60, 51, and 30% for 50, 100, and 200 mg L^{-1} gallic acid, respectively, and the flavonoid consumption was 39, 32, and 21% for 100, 200, and 400 mg L^{-1} catechin, respectively.

Comparative Study of the Effect of Phenolic Compounds in Different Culture Media. Tubes containing FT80 and MRS media supplemented with 100 mg L^{-1} gallic acid (Figure 3a) or 200 mg L^{-1}

Table 2. Consumption of Gallic Acid and Catechin in MRS Media by *L. hilgardii* 5w

	MRS + gallic acid at			MRS + catechin at		
time (h)	50 mg L^{-1}	100 mg L^{-1}	200 mg L^{-1}	100 mg L^{-1}	200 mg L^{-1}	400 mg L^{-1}
4	5.0[a]	8.0	12.0	5.0	10.0	16.0
8	15.0	22.0	24.0	11.0	24.2	40.0
12	20.5	27.6	40.0	36.5	33.6	60.0
24	30.0	50.8	60.0	39.0	63.5	83.2

[a] Results are expressed as mg L^{-1} of phenolic compounds consumed. Each value is the mean of three replicates that are reproducible within ±5%.

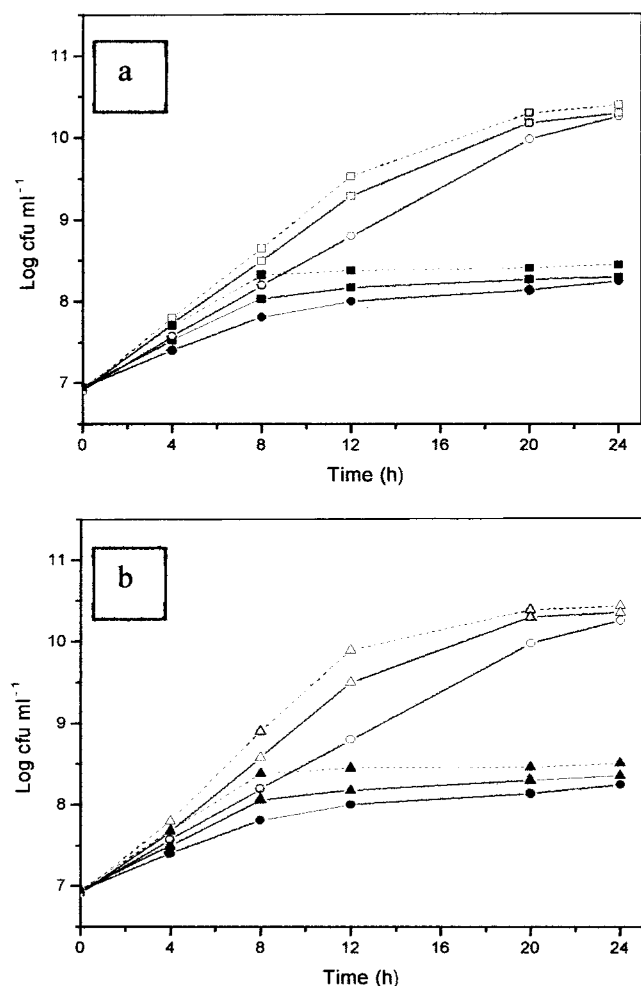

Figure 3. Influence of 100 mg L^{-1} gallic acid (a) or 200 mg L^{-1} catechin (b) on *L. hilgardii* 5w growing in FT80 media (solid symbols) and MRS media (open symbols) when cells used as inoculum were cultivated either in medium supplemented with these same concentrations of phenolic compounds (- - -) or in medium without phenolic compounds (—). Growth curves in the absence of phenolic compounds are also shown (●, ○). Values are an average of three replicates with maximum standard deviation of <5%.

catechin (Figure 3b) were inoculated with bacteria grown with or without these substrates. In FT80 medium 100 mg L^{-1} gallic acid and 200 mg L^{-1} catechin stimulated the growth of *L. hilgardii* with μ_{max} values from 0.23 h^{-1} (control) to 0.29 and 0.33 h^{-1} for gallic acid and catechin, respectively. During the stationary phase the cell density increased from 1.8 × 10^8 to 2.0 × 10^8 and 2.3 × 10^8 cfu mL^{-1} for 100 mg L^{-1} gallic acid and 200 mg L^{-1} catechin, respectively. When the cells were adapted in the media with phenolic compounds, μ_{max} values increased from 0.29 to 0.37 h^{-1} and from

Table 3. Influence of 100 mg L^{-1} Gallic Acid and 200 mg L^{-1} Catechin on Glucose Consumption by *L. hilgardii* 5w

	glucose consumption (g L^{-1}) at incubation time of				
culture medium	0 h	4 h	8 h	12 h	24 h
FT80	5.0[a]	4.9	3.4	1.1	0
+ gallic acid	5.0	4.3	3.1	0.9	0
+ gallic acid[b]	5.0	4.0	2.9	0.8	0
+ catechin	5.0	4.3	3.0	0.9	0
+ catechin[b]	5.0	3.8	2.4	0.7	0
MRS	20.0	19.7	18.7	13.6	0.2
+ gallic acid	20.0	17.3	16.3	11.5	0.2
+ gallic acid[b]	20.0	17.0	15.0	10.5	0.2
+ catechin	20.0	17.0	15.3	12.6	0.2
+ catechin[b]	20.0	16.2	14.0	11.9	0.2

[a] Each value is the mean of three replicates that are reproducible within ±3%. [b] Phenolic compounds pregrown cells.

Table 4. Influence of 100 mg L^{-1} Gallic Acid and 200 mg L^{-1} Catechin on Fructose Consumption by *L. hilgardii* 5w in FT80 Media

	fructose consumption (g L^{-1}) at incubation time of				
culture medium	0 h	4 h	8 h	12 h	24 h
FT80	3.5[a]	3.3	1.6	0.5	0.1
+ gallic acid	3.5	2.8	1.5	0.6	0.1
+ gallic acid[b]	3.5	2.5	1.4	0.5	0.1
+ catechin	3.5	2.9	1.5	0.5	0.1
+ catechin[b]	3.5	2.6	1.2	0.5	0.1

[a] Each value is the mean of three replicates that are reproducible within ±3%. [b] Phenolic compounds pre-grown cells.

0.33 to 0.41 h^{-1} and cell densities increased from 2.0 × 10^8 to 2.8 × 10^8 cfu mL^{-1} and from 2.3 × 10^8 to 3.3 × 10^8 cfu mL^{-1} for gallic acid and catechin, respectively.

In MRS media the growth rates were $\mu_{control}$ = 0.36 h^{-1}, $\mu_{gallic\ acid}$ = 0.45 h^{-1}, and $\mu_{catechin}$ = 0.52 h^{-1}. The final biomasses were 1.8 × 10^{10} cfu mL^{-1} for the control and 2.0 × 10^{10} and 2.3 × 10^{10} cfu mL^{-1} in the presence of gallic acid and catechin, respectively. When the cells were pregrown with these phenolic compounds, the μ_{max} values were 0.51 and 0.58 h^{-1} for gallic acid and catechin and the number of viable cells also increased to 2.5 × 10^{10} and 2.7 × 10^{10} cfu mL^{-1} for gallic acid and catechin, respectively.

Sugars Utilization. Table 3 shows the effect of gallic acid and catechin on glucose consumption by *L. hilgardii* 5w growing in different culture media.

In the early exponential phase of incubation (4 h) the consumption rate of glucose in FT80 media was 0.17 g L^{-1} h^{-1} in the presence of 100 mg L^{-1} of gallic acid or 200 mg L^{-1} of catechin against 0.03 g L^{-1} h^{-1} for the control. In cells pregrown with phenolic compounds the consumption rate of glucose increased from 0.17 to 0.25 and 0.30 g L^{-1} h^{-1} for the phenolic acid and the flavonoid, respectively. In MRS media the glucose consumption rate increased from 0.07 to 0.67 and 0.75 g L^{-1} h^{-1} in the presence of gallic acid and catechin, respectively. The stimulation increased more yet in cells adapted in media with gallic acid (0.75 g L^{-1} h^{-1}) and catechin (0.95 g L^{-1} h^{-1}). Despite the cited differences, at 24 h of incubation glucose was completely consumed independent of the culture medium.

As FT80 media possess fructose in their compositions, we studied the influence of gallic acid and catechin on fructose consumption by *L. hilgardii* 5w growth (Table 4). In the first hours of incubation the consumption rates of fructose were 0.05 g L^{-1} h^{-1} for the control and 0.18 and 0.15 g L^{-1} h^{-1} in the presence of gallic acid and

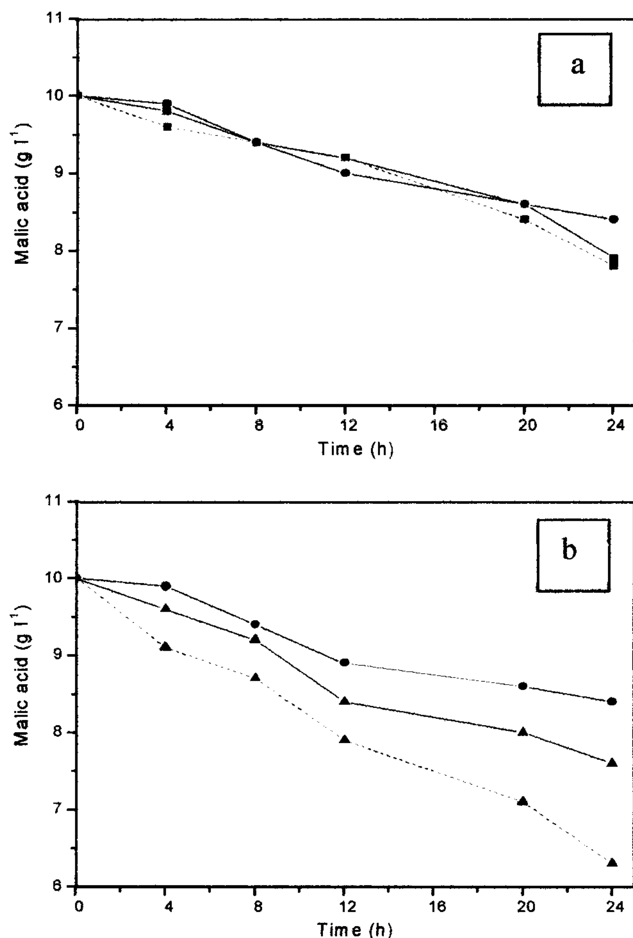

Figure 4. Influence of (a) 100 mg L^{-1} gallic acid (■) and (b) 200 mg L^{-1} catechin (▲) on malic acid consumption in FT80 medium. The cultures were inoculated with bacteria pregrown with (- - -) and without (—) the tested phenolic compounds. The growth curve in the absence of phenolic compound is also shown (●). Each value is the mean of three replicates that are reproducible within ±3%.

catechin, respectively. This effect was higher in phenolic compounds pregrown cells (0.25 and 0.23 g L^{-1} h^{-1} for gallic acid and catechin, respectively).

L-Malic Acid Utilization. L-Malic acid utilization after 24 h was higher (2.4 g L^{-1}) in the presence of catechin than in the control (1.6 g L^{-1}) (Figure 4). This effect was increased in cells adapted to the flavonoid (3.7 g L^{-1}). Gallic acid did not have any effect on malic acid utilization until 20 h of incubation. After this time, malic acid consumption rates were similar for cells grown with and without the phenolic acid (2.2 g L^{-1}).

Phenolic Acid and Flavonoid Consumption in Different Culture Conditions. The phenolic compounds consumption by *L. hilgardii* 5w is shown in Figure 5. During the first hours of incubation the gallic acid and catechin consumption rates in FT80 medium corresponded to 1.07 and 1.50 mg L^{-1} h^{-1}, respectively. These values increased when cells were precultivated in phenolic compounds (2.50 and 3.96 mg L^{-1} h^{-1} for gallic acid and catechin, respectively). The phenolic compounds consumed in 24 h were 50.1 and 43.7 mg L^{-1} for cells pregrown with and without gallic acid, respectively, and 69.4 and 57.8 mg L^{-1} for cells pregrown with and without catechin, respectively. In MRS medium the gallic acid and catechin consumption rates during the first hours of incubation corresponded to 2.75 and 3.03 mg L^{-1} h^{-1} and were not modified when the

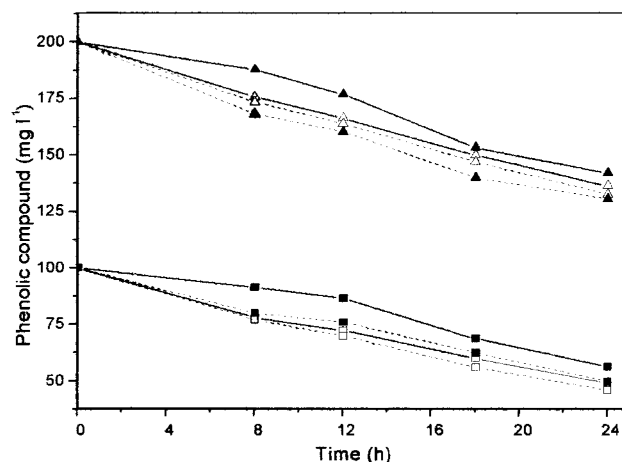

Figure 5. Gallic acid (■, □) and catechin (▲, △) consumption in MRS medium (open symbols) and FT80 (solid symbols). The cultures were inoculated with bacteria pregrown with (- - -) and without (—) the tested phenolic compounds. Each value is the mean of three replicates that are reproducible within ±5%.

cells were pregrown in phenolic compounds. In this culture medium there was no significant difference in the phenolic compounds consumed by cells pregrown with or without the phenolic compounds.

DISCUSSION

Gallic acid and catechin at concentrations normally present in wine activated *L. hilgardii* 5w growth phase and increased slightly the final biomass; these effects were augmented in cells precultivated in both phenolic compounds. In FT80 medium the growth rate activation by both phenolic compounds was higher than in MRS plus 15% tomato juice medium. The growth rate stimulation by gallic acid and catechin and the increase in cell density during the later stage of cell incubation could be related to their ability to metabolize these phenolic compounds. Stead (*7*), Vivas et al. (*8*), and Reguant et al. (*9*) also found gallic acid stimulation on lactic acid bacteria. Krumholz et al. (*22*) proposed a pathway for energy metabolism of gallate by *Eubacterium oxidoreducens*, the initial step of which is the decarboxylation of the ring. Kersten et al. (*23*) proposed a reaction sequence for the degradation by *Pseudomona testosteroni* of gallic acid, producing pyruvate and oxalacetate.

The inhibitory effect observed with high gallic acid concentrations (1000 mg L^{-1}) suggested the toxic effect on *L. hilgardii* growth at this concentration. Cornu et al. (*24*) suggested that phenolic compounds could have an activating or inhibiting effect according to their constitution and concentration and the bacterial strain used.

Using the Singleton and Rossi method, we demonstrated that *L. hilgardii* consumed these phenolic compounds. Reguant et al. (*9*) did not find modification in the UV spectra after incubation of *Œ. oeni* in the presence of different phenolic compounds. Nevertheless, Vivas et al. (*8*) reported that gallic acid disappeared during the growth of *Œ. oeni*. Cavin et al. (*10*) reported that *Lactobacillus* and *Pediococcus* strains were able to metabolize phenolic acids during growth in the FT80 medium.

Malic acid consumption rate was activated by catechin and was slightly influenced from 20 h of incubation by

gallic acid. These results on *L. hilgardii* suggested that the higher antioxidant capacity of catechin with respect to gallic acid is responsible for the increase of malic acid consumption. Reguant et al. (*9*) reported that catechin and quercetin are beneficial for *Œ. oeni* malolactic activity and that no effect was observed in the presence of gallic acid. The authors support these results considering that phenolic compounds serve as oxygen scavengers and reduce the redox potential of wines. This property and the fact that lactic acid bacteria grow better in oxygen-free culture may enhance the degradation of the malic acid.

In the presence of phenolic compounds the glucose and fructose consumption rates were higher than in the control. Sugars and malic acid could supply cells additional energy for transport and metabolism of phenolic compounds.

In FT80 medium there was a higher increase in the consumption rate of both phenolic compounds in cells preadapted than in cells nonadapted. In MRS medium there was no significant difference in the phenolic compounds consumption rate. MRS medium contained tomato juice, which included phenolic compounds in its composition. Thus, the cells grown in MRS media have an initial adaptation to phenolic compounds.

As stimulating agents of *L. hilgardii* 5w growth, gallic acid and catechin could increase the risk of spoilage lactic acid bacteria in wine. This property must be considered in the selection of the mechanism to control microbial spoilage in wines.

LITERATURE CITED

(1) Kanner, J.; Frankel, E.; Granit, R.; German, B.; Kinsella, J. E. Natural antioxidants in grapes and wine. *J. Agric. Food Chem.* **1994**, *42*, 64−69.
(2) Frankel, E. N.; Waterhouse, A. L.; Teissedre, L. P. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.
(3) Zoecklein, B. W.; Fuelsang, K. C.; Gump, B. H.; Nury, F. S. Phenolic compounds and wine color. In *Production Wine Analysis*; Van Nostrand Reinhold: New York, 1990; pp 129−168.
(4) Amerine, M. A.; Ough, C. S. Phenolic compounds. In *Methods of Analysis of Musts and Wines*; Wiley: New York, 1980; pp 175−199.
(5) Scalbert, A. Antimicrobial properties of tannins. *Phytochemistry* **1991**, *30*, 3875−3883.
(6) Stead, D. The effect of hydroxycinnamic acids on the growth of wine-spoilage lactic acid bacteria. *J. Appl. Bacteriol.* **1993**, *75*, 135−141.
(7) Stead, D. The effect of chlorogenic, gallic and quinic acids on the growth of spoilage strains of *Lactobacillus collinoides* and *Lactobacillus brevis*. *Lett. Appl. Microbiol.* **1994**, *18*, 112−114.
(8) Vivas, N.; Lonvaud-Funel, A.; Glories, Y. Effect of phenolic acids and anthocyanins on growth, viability and malolactic activity of a lactic acid bacterium. *Food Microbiol.* **1997**, *14*, 291−300.
(9) Reguant, C.; Bordons, A.; Arola L.; Rozés N. Influence of phenolic compounds on the physiology of *Œnococcus œni* from wine. *J. Appl. Microbiol.* **2000**, *88*, 1065−1071.
(10) Cavin, J. F.; Andioc, V.; Etievant, P. X.; Divies, C. Ability of wine lactic acid bacteria to metabolize phenol carboxylic acids. *Am. J. Enol. Vitic.* **1993**, *44*, 76−80.
(11) Barthelmebs, L.; Divies, C.; Cavin, J. F. Knockout of the *p*-coumarate decarboxylase gene from *Lactobacillus plantarum* reveals the existence of two other inducible enzymatic activities involved in phenolic acid metabolism. *Appl. Environ. Microbiol.* **2000**, *66*, 3368−3375.
(12) Barthelmebs, L.; Divies, C.; Cavin, J. F. Molecular characterization of the phenolic acid metabolism in the lactic acid bacteria *Lactobacillus plantarum*. *Lait* **2001**, *81*, 161−171.
(13) Strasser de Saad, A. M.; Manca de Nadra, M. C. Isolation and identification of the lactic acid bacteria from Cafayate (Argentina) wines. *Microbiol. Aliment. Nutr.* **1987**, *5*, 45−49.
(14) Rodriguez, A. V.; Manca de Nadra, M. C. Effect of pH and hydrogen peroxide produced by *Lactobacillus hilgardii* on *Pediococcus pentosaceus* growth. *FEMS Microbiol. Lett.* **1995**, *128*, 59−62.
(15) Rodriguez, A. V.; Manca de Nadra, M. C. Production of hydrogen peroxide by *Lactobacillus hilgardii* and its effect on *Leuconostoc oenos* growth. *Curr. Microbiol.* **1995**, *30*, 23−25.
(16) Farías, M. E.; Manca de Nadra, M. C.; Rollan, G. C.; Strasser de Saad, A. M. Histidine decarboxylase production by *Lactobacillus hilgardii*: Effect of organic acids. *Curr. Microbiol.* **1995**, *31*, 15−18.
(17) Farías, M. E.; Strasser de Saad, A. M.; Manca de Nadra, M. C. Viability and histidine decarboxylase activity of *Lactobacillus hilgardii* 5w during storage. *Microbiol. Aliment. Nutr.* **1996**, *14*, 351−355.
(18) De Man, J. C.; Rogosa, M.; Sharpe, M. R. A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* **1960**, *23*, 130−135.
(19) Cavin, J. F.; Schmitt, P.; Arias, A.; Lin, J.; Divies, C. Plasmid profiles in *Leuconostoc* species. *Microbiol. Aliment. Nutr.* **1988**, *6*, 55−62.
(20) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(21) Roe, J. H. A colorimetric method for the determination of fructose in blood and urine. *J. Biol. Chem.* **1934**, *107*, 15−17.
(22) Krumholz, L. R.; Crawford, R. L.; Hemling, M. E.; Bryant, M. P. Metabolism of gallate and phloroglucinol in *Eubacterium oxidoreducens* via 3-hydroxy-5-oxohexanoate. *J. Bacteriol.* **1987**, *169*, 1886−1890.
(23) Kersten, J. P.; Dagley, S.; Whittaker, J. W.; Arciero, D. M.; Lipscomb, J. D. 2-Pyrone-4,6-dicarboxylic acid, a catabolite of gallic acids in *Pseudomonas* species. *J. Bacteriol.* **1982**, *152*, 1154−1162.
(24) Cornu, M. C.; Marchand, A.; Meurville, E.; Belin, J. M. Incidences des composes phénoliques sur des bactéries lactiques et acétiques isolées du vin. *Sci. Aliment.* **1984**, *4*, 73−79.

Received for review February 15, 2001. Revised manuscript received June 12, 2001. Accepted June 12, 2001. This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT), Argentina.

JF0101915