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Some Contributions to the Study of Oenological Lactic Acid Bacteria through Their Interaction with Polyphenols

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Abstract: Probiotic features and the ability of two oenological lactic acid bacteria strains (*Pediococcus pentosaceus* CIAL-86 and *Lactobacillus plantarum* CIAL-121) and a reference probiotic strain (*Lactobacillus plantarum* CLC 17) to metabolize wine polyphenols are examined. After summarizing previous results regarding their resistance to lysozyme, gastric juice and bile salts, the three strains were assessed for their ability to release phenolic metabolites after their incubation with a wine phenolic extract. Neither of the two bacteria were able to metabolize wine polyphenols, at least in the conditions used in this study, although a certain stimulatory effect on bacterial growth was observed in the presence of a wine-derived phenolic metabolite (i.e., 3,4-dihydroxyphenylacetic acid) and a wine phenolic compound (i.e., (+)-catechin). Bacteria cell-free supernatants from the three strains delayed and inhibited almost completely the growth of the pathogen *E. coli* CIAL-153, probably due to the presence of organic acids derived from the bacterial metabolism of carbohydrates. Lastly, the three strains showed a high percentage of adhesion to intestinal cells, and pre-incubation of Caco-2 cells with bacteria strains prior to the addition of *E. coli* CIAL-153 produced a notable inhibition of the adhesion of *E. coli* to the intestinal cells.

Keywords: wine; lactic acid bacteria; probiotics; polyphenols; cell adhesion; *E. coli*; phenolic metabolism

1. Introduction

Probiotics are live microorganisms that promote healthy gastrointestinal microbiota and boost the immune response [1]. The main probiotic strains belong to the genera *Lactobacillus* and *Bifidobacterium* and were mainly isolated from dairy products or the human gastrointestinal tract. Some studies have evaluated the probiotic potential of bacterial strains isolated from alcoholic fermented beverages such as cider [2] and wine [3]. Recently, the probiotic features of strains of lactic acid bacteria (LAB) from an oenological bacteria collection, including *Lactobacillus* spp., *Pediococcus* spp. and *Oenococcus oeni*, have been assessed, although their mode of action is still poorly understood [4]. On the other hand, consumption of probiotics, which are able to metabolize polyphenols into physiologically active metabolites, has been proposed as a nutritional approach to improve the bioavailability of these phytochemicals, which would, in turn, enhance the health effects attributed to them [5,6].

Beneficial health effects derived from the moderate consumption of wine and its bioactive compounds, especially polyphenols, have been evidenced mainly in relation to diseases associated

with oxidative stress and inflammation [7,8]. Currently, the beneficial effects of wine polyphenols on intestinal microbiota growth and functionality is a topic that is attracting research [9,10]. Wine polyphenols include benzoic and cinnamic acids, phenolic alcohols and stilbenes among the non-flavonoids and anthocyanins, and flavan-3-ols, flavonols and others among the flavonoids. Most of them are minimally absorbed in the small intestine but they are extensively metabolized by enzymes from the colonic microbiota [11,12]. As colonic catabolites could be present in higher concentrations than the parent compounds, the biological activities attributed to polyphenols seem to be mainly due to them [13,14]. Therefore, the bioactivity of wine polyphenols is likely to be dependent on the microbiota activity that shows great human inter-individual differences [12].

On the other hand, numerous studies seem to indicate that phenolic compounds could positively modulate gut microbiota through prebiotic effects either promoting the growth of beneficial bacteria or having antimicrobial activity against pathogenic intestinal bacteria [15]. For instance, grape seed extracts of different flavan-3-ol composition have shown to promote the growth of potentially beneficial bacteria (*Lactobacillus* sp.) and decreased undesirable bacteria such as clostridia after batch culture fermentations [16]. Phenolic compounds contained in a cocoa powder reduced the growth of some members of the genera *Staphylococcus* and *Clostridium*, affecting the intestinal microbiota profile [17].

In this paper, we aimed to investigate more deeply the properties of potentially probiotic wine-isolated LAB. Thus, the objectives were: (a) to assess whether LAB were able to degrade wine polyphenols with the subsequent release of phenolic metabolites; (b) to monitor LAB growth in the presence and absence of some wine-related phenolic compounds; and (c) to evaluate LAB adherence to human intestinal cells, also considering the potential inhibition of the adherence of a pathogen *E. coli* strain.

2. Materials and Methods

2.1. Materials and Reagents

(+)-Catechin and 3,4-dihydroxyphenylacetic acid were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). A commercial wine phenolic extract (Provinols™) was kindly provided by Safic-Alcan (SAU, Barcelona, Spain). Its total phenolic content was 474 mg of gallic acid per gram, and the main phenolic compounds present in the extract were: anthocyanins (delphinidin-3-*O*-glucoside, 0.568 mg/g; cyanidin-3-*O*-glucoside, 0.265 mg/g; petunidin-3-*O*-glucoside, 1.47 mg/g; peonidin-3-*O*-glucoside, 1.78 mg/g; malvidin-3-*O*-glucoside, 9.01 mg/g; malvidin-3-*O*-(6"-acetyl) glucoside, 1.92 mg/g; and malvidin-3-*O*-(6"-p-coumaroyl)glucoside, 1.24 mg/g), flavan-3-ols (catechin, 9.90 mg/g; epicatechin, 6.87 mg/g; epicatechin-3-*O*-gallate, 0.226 mg/g; procyanidin B1, 11.1 mg/g; and procyanidin B2, 4.69 mg/g), benzoic acids (gallic acid, 1.06 mg/g), cinnamates (cutaric acid, 2.00 mg/g; and caftaric acid, 0.192 mg/g), flavonols (quercetin, 22.4 mg/g; kaempherol, 2.71 mg/g; myricetin, 2.55 mg/g; quercetin-3-*O*-glucoside, 0.137 mg/g; and quercetin-3-*O*-galactoside, 0.107 mg/g), stilbenes (resveratrol, 0.427 mg/g; and resveratrol-3-*O*-glucoside, 9.17 mg/g) and others (tyrosol 18.9 mg/g) [18]. The wine extract was dissolved (0.6 mg/mL) in a saline solution (NaCl 0.9%) supplemented with 0.5 g/L of cysteine and sterilized by filtration.

Formic acid was purchased from Scharlau (Scharlau, Barcelona, Spain). Acetonitrile (HPLC-MS grade) was purchased from Labscan (POCH S.A., Gliwice, Poland). Ultrapure water was obtained using a Milli-Q system (Waters Millipore, Milford, MA, USA).

2.2. LAB Strains and E. coli Strain

Two LAB strains (*P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121), previously isolated from wines [19,20], were selected based on their good probiotic features [4]. Also, a previously characterized probiotic LAB strain isolated from breast milk (*L. plantarum* CLC 17) [21] was included as a reference probiotic strain. The strain *E. coli* CIAL-153 was isolated from human faeces [4].

All strains were kept frozen at −70 °C in a sterilized mixture of culture medium and glycerol (80:20, *v*/*v*). Inocula of the three LAB strains and *E. coli* CIAL-153 strain (10^8 CFU/mL) were prepared by growing overnight the contents of thawed cryovials in MRS broth (Pronadisa, Madrid, Spain) at 30 °C and TSB broth (Scharlau, Barcelona, Spain) at 37 °C, respectively. Bacteria were harvested by centrifugation (10,000× *g*, 10 min, 4 °C) and resuspended in Dulbecco's phosphate-buffered saline DPBS (DPBS Lonza Walkersville, Inc., USA).

In vitro analyses were previously carried out to evaluate the resistance of LAB strains to conditions in the gastrointestinal tract including saliva and acid resistance, and bile tolerance [4], and the data obtained are now summarized in Table 1. All strains showed great resistance to lysozyme (>51%) and capacity to survive at low pH values (pH 1.8), thereby suggesting good adaptation of the wine LAB strains to the hostile gastrointestinal environment. Moreover, the growth percentages of both oenological LAB strains at the maximum concentration of bile assayed (1%) were higher than 84%, which was even greater than that exhibited by the reference probiotic strain, *L. plantarum* CLC 17 (73%), which reflected good bile resistance.

Table 1. Resistance to lysozyme (% Survival), tolerance to simulated gastric juice on the counts (log CFU/mL) at different pH values and incubation times, and bile resistance (% Growth) of the lactic acid bacteria (LAB) strains used in this paper [4].

	Experimental Conditions	Time (min)	LAB strains		
			***P. pentosaceus* CIAL-86**	***L. plantarum* CIAL-121**	***L. plantarum* CLC 17**
Resistance to lysozyme (% survival)		30	93.9	65.1	100
		120	88.6	50.8	86.0
Tolerance to gastric juice (log CFU/mL)	pH 5.0	0	8.47 ± 0.05	8.08 ± 0.07	8.08 ± 0.07
	pH 4.1	20	8.38 ± 0.11	8.06 ± 0.08	8.19 ± 0.10
	pH 3.0	40	8.41 ± 0.04	8.05 ± 0.13	8.08 ± 0.11
	pH 2.1	60	7.95 ± 0.06	6.20 ± 0.28	7.95 ± 0.06
	pH 1.8	90	5.15 ± 0.21	5.05 ± 0.38	7.31 ± 0.12
Bile resistance (% growth)	0.06%		99.1	93.6	93.8
	0.125%		100.0	91.2	89.1
	0.25%		88.3	89.0	77.9
	0.5%		89.5	89.5	76.8
	1.0%		84.1	88.7	73.0

Average values from three independent repetitions are presented.

2.3. Incubations of LAB Strains with Wine Polyphenols

Incubations of bacteria with wine phenolic extract (Provinols™) were carried out as previously described [22]. Briefly, 1 mL of the wine extract solution (0.6 mg/mL) was mixed with 9 mL of inocula of each LAB strain (10^8 CFU/mL) or sterile saline solution (control). Mixtures of each LAB strain suspension and saline solution (blank) (9:1) were also prepared. The mixtures were incubated at 0, 6 and 24 h, in duplicate, under anaerobic conditions at 37 °C with continuous stirring. Samples were centrifuged (10,000 rpm, for 10 min at 4 °C), and supernatants were kept at −20 °C until undergoing UPLC analyses, which were performed in duplicate.

Phenolic compounds were analysed by UPLC-ESI-MS/MS following the method of Sánchez-Patán et al. [23] with some modifications in the composition of the mobile phases: (a) water/formic acid (99.9:0.1, *v*/*v*) and (b) acetonitrile/formic acid (99.9:0.1, *v*/*v*). The MS/MS parameters (cone voltage, collision energy and MRM transition) of the 64 phenolic compounds targeted in the present study (mandelic acids, benzoic acids, phenols, hippuric acids, phenylacetic acids, phenylpropionic acids, cinnamic acids, 4-hydroxyvaleric acids, valerolactones and flavan-3-ols) were previously reported [24]. All metabolites were quantified using the calibration curves of their corresponding standards.

2.4. Growth of LAB Strains in the Presence of Phenolic Compounds

Bacterial growth was performed using the method of García-Ruiz et al. [25], slightly modified. Aliquots of 100 µL of (+)-catechin or 3,4-dihydroxyphenylacetic acid solutions (0, 100, 200 and 500 µM) were placed in microplate wells with 100 µL of culture medium (MRS). Then, 20 µL of the diluted LAB strain (inoculum of 1×10^6 CFU/mL) were added to all the microplate wells. The microtitre plates were incubated at 37 °C for 24 h in a Biotek Synergy H1™ multi-mode microplate reader (Winooki, VT, USA). Bacterial growth was determined by reading the absorbance at 600 nm. Assays were conducted twice in triplicate.

2.5. Growth of Pathogen E. coli in the Presence of Free Supernatants (CFS) from LAB Strains

Cell-free supernatants (CFS) of each LAB strain were collected from overnight cultures centrifuged at 4500 rpm for 10 min. After measuring the pH of the CFS (ranging from 5.1 to 5.4), aliquots were taken and adjusted to pH 7 using 1 M NaOH solution. All supernatants were sterilized by filtration (Symta, 0.22 µm PVDF 17 mm pK100). The bacterial growth in the presence of the CFS was measured using the microtitre assay described above. Aliquots of 200 µL of culture medium (MRS), CFS or neutralized CFS were placed in microplate wells. Then, 20 µL of the diluted *E. coli* strain (inoculum of 1×10^6 CFU/mL) were added to all the microplate wells. Assays were conducted twice in triplicate.

2.6. Cell Culture Assays: LAB Adhesion and Inhibition of E. coli Adherence to Caco-2 Cells

Caco-2™ cells from human colon adenocarcinoma (Caco-2™ ATCC®) were grown and maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma-Aldrich), supplemented with 10% (*v*/*v*) foetal calf serum at 37 °C in a 5% CO_2/95% air atmosphere. For the adhesion and inhibition experiments, Caco-2 cells were seeded in 24-well tissue plates at 25,000 cells/m^2 density and grown over 15 days to obtain a monolayer of differentiated and polarized cells, as previously described by García-Ruiz et al. [4]. Cell culture assays were performed in duplicate and three independent experiments were carried out.

To assess the adhesion of the LAB strains to Caco-2 cells, 0.5 mL of inocula of the LAB strains (10^8 CFU/mL) was added to Caco-2 cell monolayers previously washed with PBS. After 1 h of incubation at 37 °C in a 5% CO_2 atmosphere, the wells were gently washed three times with PBS solution to remove unbound bacteria. Caco-2 cells and adhered bacteria were then detached using a 0.05% trypsin-EDTA solution and bacteria counts were carried out on MRS Agar medium as described above. The adhesion capacity was expressed as the number of adhered bacteria (CFU/mL) relative to the total number of bacteria added initially (% Adhesion = (Adhered bacteria/Total of added bacteria) × 100).

In order to study the effects of LAB on the adhesion of *E. coli* to Caco-2 cells, two different experiments were carried out: (a) inhibition, to test the ability of the LAB strains to inhibit the adhesion of *E. coli*; and (b) competition, to test the ability of the LAB strains to compete with *E. coli* for adhesion to Caco-2 cells. For the inhibition experiments, LAB suspension (10^8 CFU/mL) was firstly added to Caco-2 cell monolayers, and after 1 h of incubation non-bound bacteria were removed and *E. coli* suspension (10^8 CFU/mL) was added to the wells, and the mixture was again incubated for 1 h. Caco-2 cells and adhered bacteria (LAB/*E. coli*) were then detached and *E. coli* counting was carried out on TSA plates. The inhibition of the adhesion of *E. coli* was expressed as a percentage using the following formula: Inhibition of adhesion = $100 \times (1 - T1/T2)$, where $T1$ and $T2$ are the percentage of adhesion by *E. coli* cells in the presence and absence of LAB strains, respectively. The same experimental protocol was carried out for the competition experiments, but adding the LAB and *E. coli* strains simultaneously (in an initial ratio of 1:1) to the Caco-2 cells followed by incubation for 1 h. Non-bound bacteria were removed and the bacterial counts were carried out as described above.

2.7. Statistical Analysis

A paired-sample *t*-test was used to evaluate whether the changes in phenolic content of the wine extract (% referred to the values at time 0) after incubations with bacteria was different from 100. Also, one-way analysis of variance (ANOVA) and Tukey test (at $p < 0.05$) were used for the comparison of the mean values of the LAB growth in each time for each one of time-course graphs. The IBM SPSS program for Windows was used for data processing.

3. Results and Discussion

The selected LAB strains (two oenological LAB strains *P. pentosaceus CIAL-86* and *L. plantarum* CIAL-121 and reference strain *L. plantarum* CLC 17) were used in the different designed experiments: incubations of LAB strains with wine polyphenols (Section 3.1.), growth of LAB strains in the presence of phenolic compounds (Section 3.2.), and growth of pathogen *E. coli* and its adherence to Caco-2 cells in the presence of LAB strains (Section 3.3. and Section 3.4.) The three LAB strains used had previously proven good probiotic features in vitro (Table 1) [4]. Other strains belonging to *Lactobacillus* and *Pediococcus* genera from different origins have also shown good probiotic properties such as tolerance to gastric conditions and bile tolerance [4,26,27].

3.1. Metabolism of Wine Polyphenols by LAB Strains

The capacity of three selected LAB strains (the probiotic *L. plantarum* CLC 17, and the oenological strains *P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121) to metabolize wine polyphenols was assessed through their incubation with a commercial wine phenolic extract (Provinols™) under nutrient-restricted culture conditions. Among different phenolic compounds targeted (mandelic acids, benzoic acids, phenols, hippuric acids, phenylacetic acids, phenylpropionic acids, cinnamic acids, 4-hydroxyvaleric acids, valerolactones and flavan-3-ols), a total of 15 compounds were previously quantified by UHPLC-MS/MS analysis [22]. Table 2 shows the data corresponding to the sum of the concentrations of individual compounds at 0, 6 and 24 h of incubation. Out of the three LAB strains tested, only *L. plantarum* CLC 17 produced significant increases in the concentration of phenolic compounds after 24 h of incubation (133.2% in relation to $t = 0$ h). Therefore, phenolic-degrading enzymatic activities might be strain-dependent (i.e., *L. plantarum* CLC 17), as other potential probiotic bacteria belonging to the same species were not active on wine polyphenols (i.e., *L. plantarum* CIAL-121). However, additional studies to shed light on the enzymatic activities of LAB will be intended to be carried out. Previous studies with the same wine phenolic extract used in this study also reported release of the same phenolic acids, after batch fermentations [18] or gastrointestinal digestion simulation [28] inoculated with human faecal microbiota.

Table 2. Sum (∑) of the concentrations (C, ppm) of individual phenolic compounds (gallic acid, caffeic acid, ferulic acid, protocatechuic acid, vanillic acid, salicylic acid, 3-*O*-methyl gallic acid, syringic acid, phthalic acid, 4-hydroxybenzoic acid, *p*-coumaric acid, (+)-catechin, (−)-epicatechin, procyanidin B1 and procyanidin B2) before and after incubation of the wine extract with LAB strains.

Samples	Concentrations of phenolic compounds				
	$t = 0$ h	$t = 6$ h		$t = 24$ h	
	∑C (ppm)	∑C (ppm)	% [a]	∑C (ppm)	% [a]
Wine extract	25.6	25.8	100.7	25.3	98.6
L. plantarum CLC 17 + wine extract	24.5	23.0	94.2	32.5	133.2 **
P. pentosaceus CIAL-86 + wine extract	28.7	27.9	97.4	26.9	93.8
L. plantarum CIAL-121 + wine extract	25.5	22.6	88.7	25.23	99.1

[a] percentage in relation to $t = 0$ h. ** Mean significantly different from 100 ($p < 0.01$) using paired-sample *t*-test.

3.2. Effects of Phenolic Compounds on the LAB Growth

In order to look more deeply into the effects of wine phenolic compounds and their metabolites on bacteria performance, the growth of the probiotic strain *L. plantarum* CLC 17 and the two oenological

LAB strains *P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121 was monitored in the presence of (+)-catechin, a main phenolic compound present in wine, and 3,4-dihydroxyphenylacetic acid, a microbial-derived phenolic metabolite whose concentration in faeces had been reported to significantly increase after moderate consumption of red wine [12]. Time-course graphs indicated a certain stimulatory effect of the growth of the three strains in the presence of 3,4-dihydroxyphenylacetic acid (Figure 1). On the other hand, the monomer (+)-catechin only promoted the growth of *L. plantarum* CLC 17 (Figure 1). Results of the one way analysis of variance (ANOVA) and Tukey test (at $p < 0.05$) did not show significant differences in the most of the mean values, except in the case of the *L. plantarum* CLC 17 in the presence of 3,4-dihydroxyphenylacetic acid (50, 100 and 250 µM) in comparison to the absence of the compound, from 6 to 24 h (Figure 1b). Therefore, our results confirmed that the chemical structure of polyphenols did indeed influence their effects on bacterial growth. In relation to this, other authors observed that flavanols with galloyl moiety ((−)-epigallocatechin, (−)-epicatechin-3-gallate and (−)-epigallocatechin-3-gallate) exhibited more activity on bacteria growth than those without galloyl moiety (catechins and (−)-epicatechin) [29]. Also, the microbial potency of polyphenols towards bacteria growth has also been reported to be dependent upon bacterial strain, species and genera [30], as we have also observed in our study.

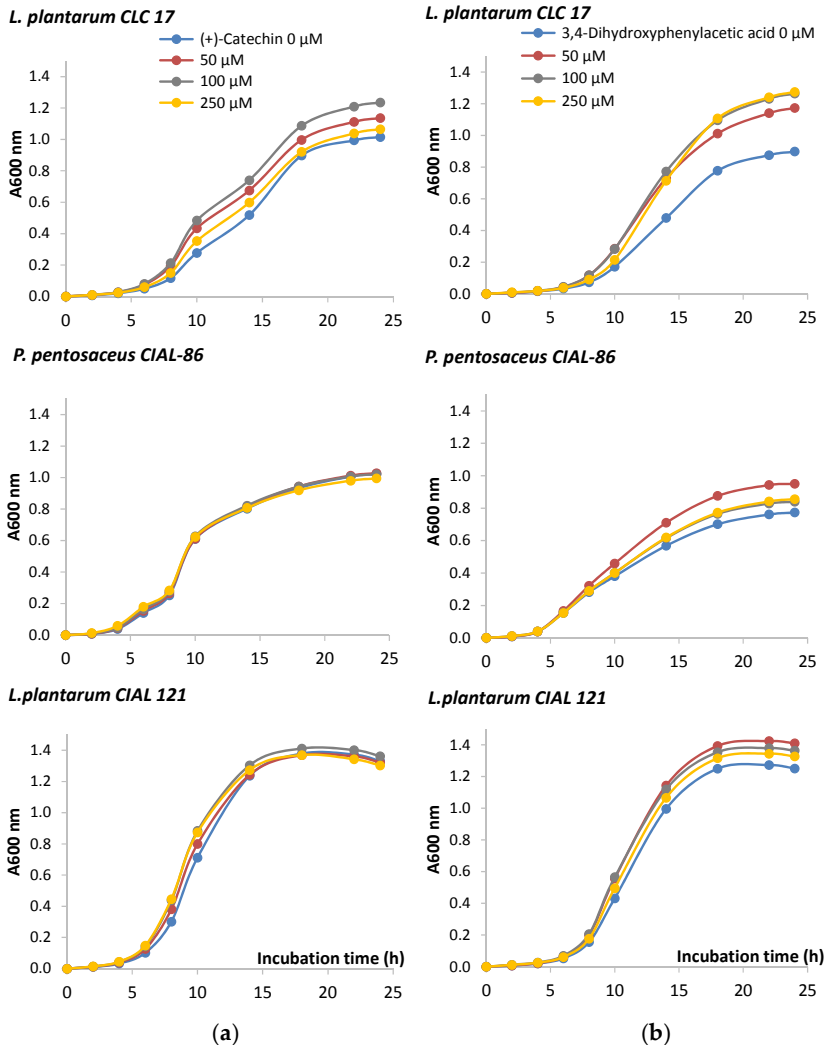

Figure 1. Growth curves of isolated LAB in the absence and presence of (+)-catechin (**a**) and 3,4-dihydroxyphenylacetic acid (**b**). The curve was developed from absorbance measures (OD:600) up to 24 h.

3.3. Effects of the Cell-Free Supernatants (CFS) from the LAB Strains on Growth of Pathogen E. coli

Bacteria cell-free supernatants of LAB strains have been reported to exhibit functions similar to the living bacteria from which they were derived, and to reduce the infection risk of the use of probiotic bacteria in patients with depressed immune systems [31]. So, CFS from the LAB strains were prepared in MRS broth and their antibacterial activity against *E. coli* CIAL-153 was evaluated (Figure 2). For the three LAB studied, CFS delayed the bacterial lag phase (from 5 to 12 h), and a strong inhibition of pathogen bacteria growth was observed (Figure 2a). Other authors have also reported an extension of the bacterial lag phase and lower growth rates of pathogen bacteria in the presence of the CFS from strains belonging to the *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Streptococcus* and *Bacillus* genera [32,33]. In agreement with them, we hypothesized that these antimicrobial effects were mainly due to the organic acids that were produced in significant quantities (and consequent lowering of pH) as a result of the ability of LAB strains to ferment carbohydrates. In addition, Gram-negatives pathogens, such as *E. coli*, tend to be more sensitive to organic acids than to bacteriocins [33], which explains the observed strong inhibition. We confirmed that the pH of the CFS from oenological LAB strains (*P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121), and the probiotic strain (*L. plantarum* CLC 17), had acidic pH values of 5.25, 5.14 and 5.11, respectively.

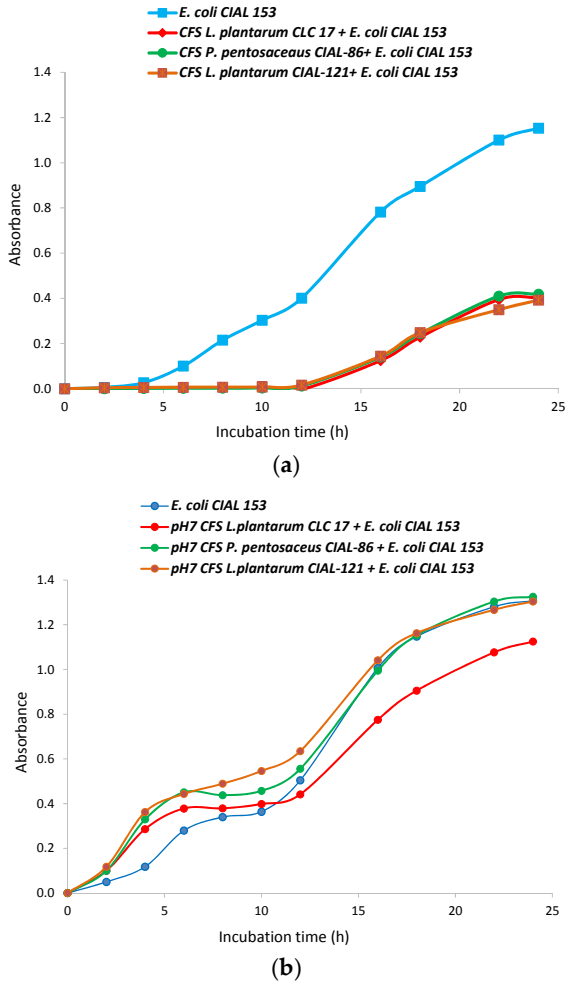

Figure 2. Growth curves of *E. coli* CIAL-153 in the presence of cell-free supernatants of *L. plantarum* CLC 17, *P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121 strains before (**a**) and after (**b**) being neutralized at pH 7.

Moreover, with the aim of researching the effect of other antimicrobial substances, such as bacteriocins, in addition to organic acids on *E. coli* growth, CFS were adjusted to pH 7 and their antibacterial activity against *E. coli* CIAL-153 was again evaluated (Figure 2b). Neutralization of the supernatants from all the LAB strains counteracted the antagonistic effects of the acid CFS against the pathogen strain, so the lag phase was similar to that of a standard growth curve and a significant increase in the growth of the pathogen was observed. Other authors have also observed that the neutralization of CFS reduced the antimicrobial activity on pathogen viability and growth, but they still observed some effects [32,33]. In our case, neutralized supernatants from the *L. plantarum* CLC 17 strain still exhibited some inhibition of the growth of *E. coli* CIAL-153, which suggested that this strain produces other antibacterial active compounds against *E. coli*. Arena et al. [34] reported that antimicrobial activity is mainly strain-specific rather than genus/species-specific and provided evidence that several of the 79 screened *L. plantarum* strains possess a significant ability to contrast various pathogenic bacteria, including both Gram-negative and Gram-positive species.

3.4. Effects of LAB on Adherence of Pathogen E. coli to Caco-2 Cells

An important property of probiotic candidates is their ability to adhere to intestinal mucosa, which excludes pathogens from cell adherence and infection progression. Initially, we investigated the ability of the three LAB strains (*L. plantarum* CLC 17, *P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121) to adhere to human intestinal Caco-2 cells because this cellular model expresses morphological and functional differentiation in vitro and shows characteristics of mature enterocytes. Adhesion levels to Caco-2 cells of the three LAB strains ranged from 8.65% to 10.01% (Figure 3) and were in line with those obtained previously [4] and in the range of other probiotics previously reported in the literature under in vitro conditions [35,36].

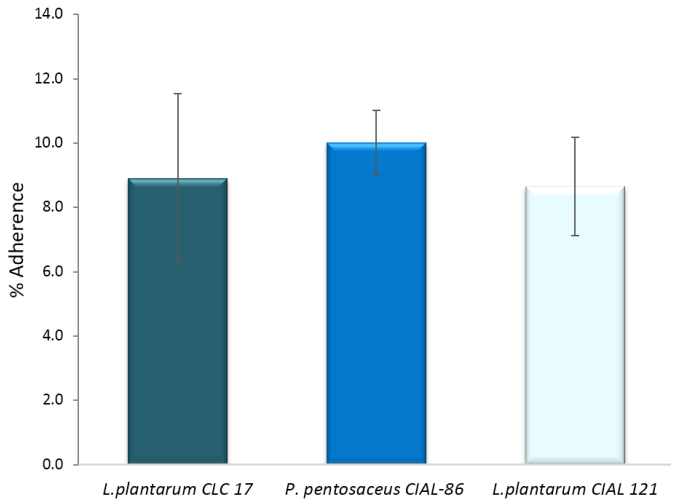

Figure 3. Adherence (%) of *P. pentosaceus* CIAL-86, *L. plantarum* CIAL-121 and *L. plantarum* CLC 17 strains to Caco-2 cells. Results are shown as media ± standard deviation.

Having confirmed the ability of LAB strains to adhere to Caco-2 cells, the adhesion of *E. coli* CIAL-153 to these intestinal cells was assessed in the presence of the different LAB strains. Initially, it was found that the adhesion of *E. coli* CIAL-153 to Caco-2 cells on their own was 8.51% ± 1.83%. The inhibition of adherence of *E. coli* CIAL-153 to Caco-2 cells for probiotic LAB strains in the anti-adhesion assays (competition and inhibition) is shown in Figure 4. Pre-incubation of Caco-2 cells with LAB strains prior to the addition of *E. coli* CIAL-153 (inhibition assay) produced a notable inhibition of the adhesion of *E. coli* to the intestinal cells for three strains in respect to the control (absence of LAB strains). *P. pentosaceus* CIAL-86 was the most effective strain in inhibiting the adhesion of *E. coli* CIAL-153 (>35%), while *L. plantarum* CIAL-121 showed similar inhibition values to

those observed in the reference probiotic strain, *L. plantarum* CLC 17 (20.7% and 22.6%, respectively) (Figure 4). These percentages were similar when Caco-2 cells were incubated at the same time with both *E. coli* and probiotic LAB strains (competition assay), ranging from 17% to 22% in respect to the control (absence of LAB strains). The high values from the inhibition experiment could indicate an effective competition of LAB strains against *E. coli* CIAL-153 for common adhesion receptors [37] or other anti-adhesion factors [38]. Thus, the ability to inhibit the adhesion of *E. coli* CIAL-153 to Caco-2 cells appeared to be influenced by LAB strains, which suggested a certain pathogen-LAB specificity as indicated by other authors [35].

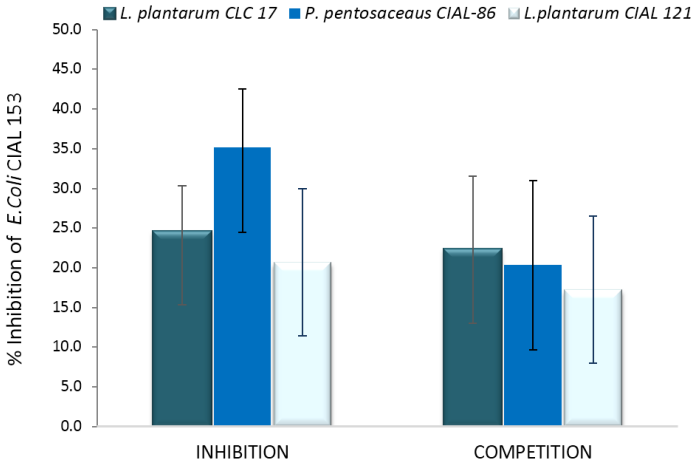

Figure 4. Inhibition (%) of adherence (%) of *E. coli* CIAL-153 to Caco-2 cells in the presence of *L. plantarum* CLC 17, *P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121 in anti-adhesion assays (competition and inhibition). Results are shown as media ± standard deviation.

4. Conclusions

In vivo reports suggest that wine polyphenols exert an essential impact on intestinal microbiota growth and functionality (see [9] for review). However, an important question that remains unsolved is whether these benefits may be enhance by the concomitant interactions by wine polyphenols and probiotics at the gut level. This paper investigates some new metabolic features and probiotic characteristics of oenological lactic acid bacteria, in particular *P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121, based on their interaction with polyphenols. Neither of these two oenological bacteria was able to metabolize wine polyphenols, at least in the conditions used in this study, although this metabolic potential migth be strain-dependent, as the probiotic reference strain *L. plantarum* CLC 17 was found to be effective in metabolizing wine polyphenols. However, growth of both oenological (*P. pentosaceus* CIAL-86 and *L. plantarum* CIAL-121) and reference (*L. plantarum* CLC 17) strains was stimulated in the presence of wine phenolic compounds (i.e., (+)-catechin) and wine-derived phenolic metabolites (i.e., 3,4-dihydroxyphenylacetic acid), although no clear dose-dependent effect was observed. Bacteria cell-free supernatants from the three LAB strains delayed and inhibited almost completely the *E. coli* CIAL-153 growth, which may be mainly attributed to the presence of organic acids derived from the metabolism of carbohydrates by LAB. In relation to their adhesion to intestinal cells, the three LAB strains showed a high adhesion percentage, especially *P. pentosaceus* CIAL-86. Moreover, pre-incubation of Caco-2 cells with LAB strains prior to the addition of *E. coli* CIAL-153 produced a notable inhibition of the adhesion of *E. coli* to the intestinal cells. Nevertheless, the effect of selected lactic acid bacteria on the growth and adhesion to intestinal cells of other gut pathogenic bacteria should be investigated. To our knowledge, there are very few reports considering probiotic features of LAB isolated from wine such as the ones investigated here, which emphasizes the novelty of these results. Overall, these in vitro results confirm the potential of oenological LAB strains as probiotics, with the aim of developing general nutritional strategies and designing specific dietary

recommendations based on the combination of active phenolic compounds/extracts and probiotics, thus contributing to the ultimate goal of promoting intestinal health. Nevertheless, further in vitro and in vivo investigations are still necessary in order to confirm these potential beneficial effects.

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