Inhibiting potential of selected lactic acid bacteria isolated from Costa Rican agro-industrial waste against *Salmonella* sp. in yogurt

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Abstract

This study aimed to characterize lactic acid bacteria (LAB) isolated from Costa Rican agro-industrial waste and explore their bioprotective potential against *Salmonella* in yogurt. A total of 43 LAB isolates were identified using the 16S rRNA region. *In vitro* inhibition of *Salmonella, Listeria monocytogenes, Staphylococcus aureus,* and *Escherichia coli* was determined. Fifteen of the 43 isolates showed a good to strong antimicrobial effect against at least two pathogens. Fourteen selected isolates were evaluated for antibiotic resistance, gelatinase, and hemolytic activity. The bioprotective effect of the most promising strain, *Lactiplantibacillus pentosus*, was assessed against *Salmonella* sp. during yogurt fermentation. All the isolates were resistant to vancomycin and showed variable degrees of susceptibility to other antibiotics. All of the isolates were negative for gelatinase, and five isolates had no hemolytic activity. A significant inhibitory effect of *L. pentosus*_58(6)-2I ($P < 0.05$) against *Salmonella* during fermentation was found, but pathogen reduction was limited to 0.611 log CFU/mL.

Introduction

In Costa Rica, more than 6.3 billion tons of organic waste are generated in the primary economic sector alone (Miranda-Durán *et al*., 2020), underscoring the urgent need for innovative waste management strategies. Agro-industrial waste is any substance or object that the holder discards or intends or is required to discard, considering residue as everything that is not the final (main) product of the process (Okino Delgado *et al*., 2015).

The isolation of lactic acid bacteria (LAB) from agro-industrial waste could be a feasible option to obtain microorganisms adapted to local conditions. Recent research (de Melo Pereira *et al*., 2018; Todorov *et al*., 2024) have been shown that bacteria isolated from sources other than the gastrointestinal tract such as strains from local foods, traditional drinks, fruits, fermented process, and agroindustry waste (Amenu *et al*., 2024; Prihanto *et al*., 2024; Santos *et al*., 2016; Taboada *et al*., 2024, Wu et al., 2021), could be an option to obtain isolates with probiotic potential. Bioprotective potential is a desired feature which can offer an additional safety barrier against pathogenic microorganisms in addition to the thermal treatments used by the food industry.

The use of LAB in fermented products can contribute to preventing food borne diseases (Martin-Garcia *et al*., 2023). Some members of the genera *Lactobacillus* and *Bifidobacterium* are characterized by the production of organic acids, specifically lactic and acetic acids, and some strains have been studied to prevent the growth of pathogenic bacteria such as *Salmonella* (Motahari *et al.*, 2017).

Salmonella spp. is one of the most frequent bacterial etiological agents of foodborne diseases in the European Union and the United States (EFSA and ECDC, 2021; Williams *et al.*, 2023). It is transmitted by the fecal-oral route, either directly or through food. Milk and milk derivative products have been implicated in the transmission of

Salmonella, mostly due to the use of raw or inadequately pasteurized milk or contaminated after pasteurization (Olsen *et al*., 2004; Singh *et al*., 2018). A survival of *S.* Typhimurium for 23 days and *S.* Typhi during 16 days in a refrigerated (4°C) Egyptian yogurt has been previously reported (El-Gazzar and Marth, 1992).

Yogurt presents unfavorable conditions for the growth of *Salmonella*, however, a research study shows a maximum specific growth rate of *Salmonella* during yogurt fermentation that ranged from 0.26 to 0.38 for *S. Enteritidis* and from 0.50 to 0.56 log CFU/g/h for *S. Typhimurium* (Savran *et al*., 2018a). Moreover, it has been confirmed that *S. Enteriditis* is able to survive longer during yogurt storage when temperatures are low (e.g. 304 h at 4 °C, 60 h at 25 °C) (Savran *et al.*, 2018b). The use of contaminated raw milk or the incorrect application of hygiene practices could be a source of contamination (Kumbhar *et al*., 2009). Despite only one outbreak of salmonellosis in yogurt has been reported associated with cross contamination due to an open, blood-stained yogurt pots stored beneath a rack of raw lamb (Evans *et al.*, 1995), recent data show the presence of *Salmonella* in raw milk, yogurt, and other dairy products (Asfaw *et al*., 2023).

This research aimed to characterize LAB isolated from Costa Rican agro-industrial residuals, and explore the bioprotective potential of a selected one against *Salmonella* during yogurt fermentation.

Materials and Methods

Isolation of LAB from Agro-industrial waste

Agro-industrial wastes were collected from Costa Rican companies that produce value-added products from coffee, pineapple, orange, coffee, cocoa, and carrot (Table 1, Supplementary Table 1). The colonies of LAB for each agro-industrial waste were obtained according to Wu *et al.* (2021). Selected colonies were identified by Gram

staining and morphology. The cultures were preserved as glycerol stocks (20% v/v) at -80 °C until examination.

DNA extraction and PCR amplification

Lactic acid bacteria were grown in MRS agar (Oxoid) for 22 ± 2 h at 35.0 ± 0.5 °C. Using a miniprep extraction protocol (Birnboim and Doly, 1979), total nucleic acids were extracted from each isolate. The primer pair 27F/1492R was used to obtain a 1.5-kb fragment of the 16S rRNA gene (Edwards *et al.*, 1989). PCR was conducted according to Wu *et al*. (2021).

Antimicrobial activity of LAB isolates against foodborne pathogens

A modified methodology of the overlay assay (Hütt *et al*., 2006; Soleimani *et al*., 2010) was used to evaluate the *in vitro* antagonistic capacity of the LAB isolates against *Salmonella, L. monocytogenes*, *S. aureus*, and *E. coli*. Microorganisms used in the study included five *L. monocytogenes* strains (ATCC 19116 and wild strains isolated from meat products), five *Salmonella* isolates (*Salmonella* Typhimurium, *Salmonella* Typhi, and three wild isolates of an undefined serotype); all of them isolated from clinical samples, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Pathogens were provided by the bacteriology collection of the Food Microbiology Research and Training Laboratory from the Faculty of Microbiology at the University of Costa Rica. In the case of *Salmonella* or *L. monocytogenes*, a cocktail suspension was used to inoculate. Before the experiments, the plates were incubated at 35.0 ± 0.5 °C for 24 ± 2 h in MRS (Oxoid) or Tryptic Soy Broth (TSB) (Oxoid), respectively. After incubation, each LAB isolate was inoculated in a straight line 7 cm long and 0.5 cm from the edge, using MRS agar. The plates were incubated under capnophilic conditions at 35.0 ± 0.5 ◦C for 24 ± 2 h. Before, 5 mL of

Brain Heart Infusion agar (BHI) (Oxoid) was added. After solidification, a cocktail suspension prepared with the overnight cultures of each pathogen was added. The Petri dishes were incubated at 35.0 ± 0.5 ◦C for 24 ± 2 h under aerobic conditions and they were examined for the presence of an antagonistic interaction between each LAB isolate and the pathogens. Antagonistic effect was visualized as a clear inhibition zone around the line of each LAB. Clear zones were measured (Pan *et al.*, 2009; Wu *et al.*, 2021; Duche *et al.*, 2023) and the isolates were classified according to the size of the inhibition zone. The 14 isolates showing the strongest inhibition halo against the pathogens were selected for safety testing.

Safety assays

Antibiotic resistance

Antibiotic resistance of the LAB isolates was evaluated. A total of nine antibiotics of the main classes were used (Table 2). Each isolate was grown in MRS broth (Oxoid) incubated at 35.0 ± 0.5 ºC for 24 ± 2 h and they were swabbed on Mueller-Hinton agar (Oxoid) using a sterile cotton swab. Disks, impregnated with each antibiotic, were placed on the agar plates that were incubated at 35.0 ± 0.5 ºC for 24 ± 2 h in capnophilic conditions. Diameter of the inhibition zones was measured after incubation and interpreted according to the standards established by the Clinical and Laboratory Standard Institute (Sharma *et al*., 2016). Experiments were performed in duplicate.

Gelatinase and hemolytic activity

The LAB isolates were grown on MRS agar (Oxoid) at 35.0 ± 0.5 ºC for 48 ± 2 h. For the gelatinase test, one colony from each isolate was inoculated into nutritive gelatin tubes and incubated for 7 days at 35.0 ± 0.5 ºC. Every 48 ± 2 h, tubes were placed

158 in an ice bath for 15 ± 2 min and observed for gelatin hydrolysis. Isolates were considered
159 gelatinase negative if the gel remained solid after 7 days of incubation, or positive if there
160 was hydrolysis (Klamm, 2019). The experiment was performed in duplicate. *S. aureus*
161 ATCC 25923 and *E. coli* ATCC 25922 were used as positive controls, and an
162 uninoculated tube as a negative control.
163 For the hemolysis test, 0.5 McFarland standard suspension was prepared for each
164 isolate in 0.1% sterile peptone water. The suspensions were streaked as pure cultures on
165 Columbia agar with 7% sheep blood and incubated at 35 °C for 48 h (Maasjost *et al*.,
166 2019). Color changes in zones on the blood agar indicated hemolytic activity: green zone
167 (α-hemolysis), light zone (β-hemolysis), and no color change (γ- hemolysis). Two
168 replicates were performed. *S. aureus* ATCC 25923 was used as a positive control (Aziz
169 *et al*., 2021).
170
171 **Bioprotective effect of *L. pentosus* against *Salmonella* sp. in yogurt**
172 **Pathogen inoculation**
173 Five *Salmonella* strains (*S. enterica* serovar Typhimurium 93, *S. enterica* 750, *S.*
174 *enterica* 80, and *Salmonella* DA36) were grown individually in TSB at 35.0 ± 0.5 ºC for
175 24 ± 2 h. Stationary phase cultures were then mixed in equal proportions. From the initial
176 *Salmonella* sp. cocktail decimal dilutions were made to obtain a population of 7 log
177 CFU/mL. Finally, 1 mL was inoculated into 1 L of yogurt targeting an initial pathogen
178 population of 4 log CFU/mL.
179
180 ***Lactiplantibacillus pentosus* inoculation**

181 *L. pentosus*_58(6)-2I was grown for 24 h in MRS broth at 35 ± 0.5 ºC. The
182 inoculum was added to the yogurt (20 mL for 2 L of product) for an initial population of
183 6-7 log CFU/mL.
184
185 **Yogurt manufacture and inoculation**
186 A formulation of 95% skim milk and 5% skim milk powder was used. The mixture
187 was pasteurized at 90 ± 2 ºC for 10 min and then cooled to 43-44 ºC. The commercial
188 culture Yo-Flex (CHR HANSEN), (*Streptococcus thermophilus* and *Lactobacillus*
189 *bulgaricus)*, was added in the amount recommended by the supplier. The yogurt was
190 divided into four portions and used in the following treatments: 1) uninoculated yogurt,
191 2) yogurt inoculated with the *Salmonella* sp. cocktail 3) yogurt supplemented with 6 log
192 CFU/mL of *L. pentosus*_58(6)-2I, and 4) yogurt supplemented with 6 log CFU/mL of *L.*
193 *pentosus*_58(6)-2I and *Salmonella* sp. All treatments were incubated at 41 ºC in 8 oz
194 containers until a pH of approximately 4.5 was reached. The assay was performed in
195 triplicate.
196
197 **Microbiological analysis**
198 Treatments were sampled hourly during 6 h of fermentation. Decimal dilutions of
199 each sample were made in 0.1% phosphate saline solution (PSA). LAB counts (including
200 *L. pentosus*_58(6)-2I) were performed with the 3M Petrifilm method whereas *Salmonella*
201 s p. was quantified on xylose-lysine/deoxycholate agar (XLD) (Oxoid) using the spread
202 plate technique. Plates were incubated at 35 ºC for 48 ± 3 h. The yogurt pH was monitored
203 in the four treatments to observe the effects of *Salmonella* sp. or *L. pentosus*_58(6)-2I on
204 the acidification curve during fermentation. The pH of the uninoculated yogurt, and of
205 the yogurt with added *L. pentosus* was measured every 30 min using a HI2002-01 edge

pH meter (Hanna Instruments, Woonsocket, RI) equipped with a HI10530 electrode (Hanna Instruments). The pH of the treatments inoculated with *Salmonella* spp. was measured every hour. The uninoculated yogurt's moisture, ash, protein and sodium contents were determined using standard AOAC International methods (AOAC International, 2012). Fat content was determined in yogurt as previously described (Carpenter *et al*., 1993), and carbohydrate content was determined by calculation.

Statistical analysis

An analysis of variance (ANOVA) was performed to determine differences between the growth or death curves (log CFU/mL) at time 0 and 6 h of *L. pentosus*_58(6)-2I and *Salmonella* sp. in the yogurt treatments. ANOVA was also performed for the acidification curves (pH values at time 0 and 6 h). A significance level of 5% was established, with values of $P<0.05$ considered significant. When significant differences were identified, an HSD-Tukey multiple comparison of means test was performed to determine the difference between treatments.

Results

At least 10 different species of LAB were identified from the agro-industrial wastes (Table 1, Supplementary Table 1). Out of 43 isolates obtained from culture, 17 showed some degree of antagonistic activity against at least one of the tested pathogens. However, just 14 isolates were selected for further trials based on their antimicrobial effect against at least two pathogens (inhibition diameter larger than 6 mm). The only exception was *L. argentoratensis*_79(4)-2C (Table 1).

For antibiotic resistance, all the selected LABs were resistant to vancomycin. *L. paracasei* subsp. *tolerans* strains were susceptible to tetracycline but they were resistant

231 to streptomycin, chloramphenicol, erythromycin and penicillin whereas the isolates *L.*
232 *plantarum*_17-(4D), *L. plantarum* subsp. *plantarum*_71-6(2F), *L. argentoratensis*_57(7)-
233 1H, and *L. argentoratensis*_79(4)-2C were resistant to ciprofloxacin (Table 2).
234 In the case of hemolytic and gelatinase activity, *L. paracasei* subsp. *tolerans*
235 (2A2-B, IA2P, II-CI-C Y 11-C1-B) and *L. casei* ATCC 393 did not produce beta
236 hemolysis and were negative for gelatinase activity (Table 3).
237
238 **Biopreservative effect of *Lactobacillus pentosus* during yogurt processing**
239 Based on the previous results, *L. pentosus*_58(6)-2I was selected as a potential
240 biopreservative for yogurt. The nutritional profile of the yogurt used is summarized in
241 Table 4. Figure 1 shows the pH of the four yogurt treatments during fermentation. The
242 acidification curves were consistent with the profile provided by the reference starter
243 culture after 6 h of fermentation at 41 °C. There were no significant differences among
244 treatments (P= 0.338).
245 Total LAB counts differed significantly (P = 0.010) among three of the treatments
246 (yogurt inoculated with *L. pentosus* and *Salmonella* sp., yogurt inoculated with
247 *Salmonella* spp., and uninoculated yogurt) after 6 h of fermentation. Specifically, there
248 were differences in bacterial counts after 6 h of fermentation, between yogurt inoculated
249 with *L. pentosus* and *Salmonella* sp. and yogurt with *Salmonella* sp. (P = 0.008) (Figure
250 2). However, these two treatments did not differ from the control (uninoculated sample)
251 (P = 0.538 and P = 0.108, respectively). As expected, the initial LAB population was
252 higher in yogurt inoculated with *L. pentosus* and *Salmonella* sp. than in yogurt inoculated
253 with *Salmonella*. However, the LAB population stabilized after 2 h of fermentation and
254 remained constant until the end of the process. This was consistent with the acidification
255 curves since the pH values did not change with the addition of *L. pentosus* (Figure 1).

Salmonella sp. survival in yogurt inoculated with *L. pentosus*_58(6)-2I was significantly lower ($P = 0.019$) compared to the control (Figure 3). However, the HSD-Tukey test did not show differences between pathogen populations at times 0 and 6 in either of the treatments ($P = 0.331$ and $P = 1.00$, respectively), and differences between the two treatments at time 6 h were not significant ($P < 0.05$). There was a pathogen reduction of 0.611 log CFU/g in the treatment with *L. pentosus*_58(6)-2I and 0.017 log CFU/g in the negative control. The *Salmonella* population increased during the initial stage of fermentation (after 2 h of fermentation in the positive control and after 3 h in the negative control). However, after a longer period of fermentation, this population decreased, especially in the presence of *L. pentosus*_58(6)-2I.

Discussion and Conclusions

L. paracasei frequently exhibit broad-spectrum antimicrobial activity with simultaneous inhibitory effects against *L. monocytogenes*, *E. coli*, *S. aureus*, and *Salmonella* (Akpinar and Yerkliyaka, 2021), that is related with the production of antimicrobial compounds such as organic acids, bacteriocins and exopolysaccharides (Amini *et al.*, 2022). The antagonistic activity of *L. argentoratensis* is closely related to *L. plantarum* and it was recently classified as a new species (McFrederick *et al.*, 2018). Literature about the antimicrobial capacity of this species is relatively scarce; however, some studies have confirmed the antimicrobial capacity of some isolates against Gram positive and Gram negative bacteria (Siangpro *et al*., 2023). Recent advances in whole genome sequencing of *L. argentoratensis* are providing insights about the potential of this species as a biocontrol agent (Syrokou *et al*., 2021).

Vancomycin resistance found in this research was similar to previous reports (Guo, 2017). This resistance is intrinsic in nature and is given by the vanX gene which

281 codes for the dipeptide ligase enzyme (Ddi) (Guo, 2017; Zhang *et al.*, 2018), and transfer
282 to foodborne pathogens is not expected (Álvarez and Poce, 2018). LAB normally have
283 more than 70% resistance to amynoglicosides (gentamicin and streptomycin) and
284 ciprofloxacin, and low resistance to penicillin, tetracycline and chloramphenicol.
285 Variability in antibiotic resistance among species may be related to intrinsic traits. For
286 example, more than 68% of Lactobacillus species are resistant to ciprofloxacin due to the
287 gyrA gene. The tet(M) and erm(B) genes of *L. paracasei* confer resistance to tetracycline
288 and erythromycin (Guo *et al*., 2017).

289 Bacteria that produce total hemolysis in agar may contribute to anemia,
290 inflammation, and edema, mostly due to decreased iron availability (Rastogi *et al*., 2021).
291 Therefore, non-hemolytic LAB strains are considered safer for food applications. Some
292 isolates from this study were classified as partial-hemolytic strains; however, this trait is
293 normal in *Lactobacillus* and it is attributed to the generation of hydrogen peroxide (Aziz
294 *et al.*, 2021). Also, no gelatinase activity was found in *Lactobacillus* (Aziz *et al*., 2021)
295 due to its low capacity to hydrolyze tissue components. This feature supports that LAB
296 strains are safe for food applications (Hashem *et al.,* 2020).

297 The pathogen reduction observed in this study in the presence of *L.*
298 *pentosus*_58(6)-2I was greater than the decrease in *Salmonella* sp. due to the effect of
299 low pH reported by Savran *et al.* (2018a). Other mechanisms not studied here that may
300 explain the pathogen reduction include the synthesis of biosurfactant compounds,
301 bacteriocins, and hydrogen peroxide, which have *in vitro* inhibitory effects against
302 *Salmonella* sp. (Liu *et al.,* 2018). Moreover, the bioprotective effect of *L. pentosus* against
303 *Salmonella* sp. was demonstrated by Motahari *et al.* (2017) using another *L. pentosus*
304 strain. Further analyses are required to elucidate the causes behind the greater decrease
305 in *Salmonella* spp. in the presence of *L. pentosus*_58(6)-2I.

The effect of a higher load of *L. pentosus*_58(6)-2I on *Salmonella* sp. survival during yogurt fermentation should be tested. If effective, this approach may be suitable if the sensorial properties of yogurt and acidification curves are not affected, and consumer acceptance is not compromised. *L. pentosus*_58(6)-2I should also be evaluated in other foods such as dairy products and fermented meats.

Likewise, other properties should be assessed to identify bacteria with probiotic potential. According to Todorov *et al*. (2023), evaluation under simulated gastrointestinal tract conditions, antagonism against pathogens, resistance to enzymes, presence of transferable antibiotic resistance genes, ability to reduce pathogen adhesion to surfaces, removal of cholesterol from surfaces, as well as taking into account the evaluation of the shelf life of foods and their sensory characteristics, are some characteristics that should be considered when evaluating the properties of new isolates.

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497 Table 1. Inhibition halo of *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus* 29213 and *Escherichia coli* 25922 grown on

498 culture media pre-inoculated with selected LAB strains isolated from agro-industrial waste.

			Halo			
LAB strain	**GenBank code**	**Isolation source**	***Salmonella***	***L. monocytogenes***	***S. aureus***	***E. coli***
Lacticaseibacillus paracasei subsp. *tolerans*_2A2-B	ON763280	MFC of coffee effluent	+++	+++	+++	+++
Lacticaseibacillus paracasei subsp. *tolerans*_ IA2-P	ON763283	MFC of coffee effluent	+++	+++	+++	+++
Lacticaseibacillus paracasei subsp. *tolerans*_1-C1	ON763284	MFC of coffee effluent	+++	+++	+++	+++
*Lacticaseibacillus paracasei subsp. tolerans*_11-CI-C	ON763282	MFC of coffee effluent	+++	+++	+++	+++
Lacticaseibacillus paracasei subsp. *tolerans*_11-C2-C	ON763287	MFC of coffee effluent	+++	+++	+	++
Lacticaseibacillus paracasei subsp. *tolerans*_11-C1-B	ON763286	MFC of coffee effluent	+++	+++	++	+++
Lacticaseibacillus paracasei subsp. *tolerans*_I-C2	ON763285	MFC of coffee effluent	+++	+++	+++	+++
*Leuconostoc pseudomesenteroides*_17-(2D)	ON763309	Coffee brush	++	+	+++	+++
*Lactiplantibacillus plantarum*_17-(4D)	ON763301	Coffee brush	+++	+++	++	+++
Lactobacillus plantarum subsp. *plantarum*_71-6(2F)	ON763308	Orange waste residuals	+++	+++	+++	+
*Lactiplantibacillus argentoratensis*_57(7)-1H	ON763326	Trinitario cocoa	+++	++	+++	+
*Lactiplantibacillus pentosus*_58(6)-2I	ON763304	Trinitario cocoa	+++	+	+	+
*Lactiplantibacillus pentosus*_58(6)-1I	ON763303	Trinitario cocoa	+++	++	++	++
*Lactiplantibacillusargentoratensis*_79(4)-2C	ON763328	Trinitario cocoa	++	++	++	+

+ Inhibition zone 0- 3 mm in diameter (weak), ++ inhibition zone 3- 6 mm in diameter (good), +++ inhibition zone larger than 6 mm in diameter (strong). MFC=microbial fuel cells.

514 Table 2. Antibiotic resistance/susceptibility of selected isolates from agro-industrial waste (S, susceptible. R, resistant. I, intermediate).

Isolate	Antibiotic (concentration)								
	Amoxicillin with clavulanic acid (30 µg)	Streptomycin (15 µg)	Chloramphenicol (30 µg)	Gentamicin (10 µg)	Erythromycin (15 µg)	Tetracycline (30 µg)	Ciprofloxacin (5 µg)	Vancomycin (30 µg)	Penicillin (10 IU)
L. paracasei subsp. *tolerans*_2A2-B	S	R	S	S	S	S	S	R	S
L. paracasei subsp. *tolerans*_IA2-P	S	R	R	S	R	R	S	R	R
L. paracasei subsp. *tolerans*_1-C1	S	S	S	S	S	S	S	R	S
L. paracasei subsp. *tolerans*_II-CI-C	S	R	S	S	S	S	S	R	S
L. paracasei subsp. *tolerans*_11-C2-C	S	I	S	S	S	S	S	R	S
L. paracasei subsp. *tolerans*_11-C1-B	S	R	S	S	S	S	S	R	S
L. paracasei subsp. *tolerans*_I-C2	S	R	S	S	S	S	S	R	S
*L. pseudomesenteroides*_17-(2D)	S	S	S	S	S	S	S	R	S
*L. plantarum*_17-(4D)	S	S	S	S	S	S	R	R	S
L. plantarum subsp. *plantarum*_71-6(2F)	S	S	S	S	S	S	R	R	S
*L. argentoratensis*_57(7)-1H	S	S	S	S	S	S	R	R	S
*L. pentosus*_58(6)-2I	S	S	S	S	S	S	I	R	S
*L. pentosus*_58(6)-1I	S	S	S	S	S	S	S	R	S
*L. argentoratensis*_79(4)-2C	S	S	S	S	S	S	R	R	S
*L. casei*_ATCC 393	S	S	R	R	R	R	S	R	S
*L. paracasei*_6714	S	R	S	S	S	S	S	R	S

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516 Table 3. Results of hemolytic activity and gelatinase activity to evaluate the probiotic
517 profile of selected lactic acid bacteria isolated from agroindustrial waste.

Isolate	Hemolytic	Gelatinase
Lacticaseibacillus paracasei subsp. *tolerans*_2A2-B	γ	Neg
Lacticaseibacillus paracasei subsp. *tolerans*_IA2-P	γ	Neg
Lacticaseibacillus paracasei subsp. *tolerans*_1-C1	α	Neg
Lacticaseibacillus paracasei subsp. *tolerans*_11-CI-C	γ	Neg
Lacticaseibacillus paracasei subsp. *tolerans*_11-C2-C	α	Neg
Lacticaseibacillus paracasei subsp. *tolerans*_11-C1-B	γ	Neg
Lacticaseibacillus paracasei subsp. *tolerans*_I-C2	α	Neg
*Leuconostoc pseudomesenteroides*_17-(2D)	α	Neg
*Lactobacillus plantarum*_17-(4D)	α	Neg
Lactobacillus plantarum subsp. *plantarum*_71-6(2F)	α	Neg
*Lactiplantibacillus argentoratensis*_57(7)-1H	α	Neg
*Lactiplantibacillus pentosus*_58(6)-2I	α	Neg
*Lactiplantibacillus pentosus*_58(6)-1I	α	Neg
*Lactiplantibacillus argentoratensis*_79(4)-2C	α	Neg
Lacticaseibacillus paracasei ATCC 393	γ	Neg
*Lacticaseibacillus paracasei*_6714	α	Neg

518 Absence of hemolysis (γ), partial hemolysis (α), negative (neg).

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Table 4. Nutritional composition of yogurt.

Analysis	Percentage (%) ± SD
Moisture	85.57 ± 0.29
Fat	<0.20 ± 0.00
Protein	4.95 ± 0.14
Ash	1.12 ± 0.10
Carbohydrates	7.32 ± 0.17
Sodium	74.30 ± 4.98
Total energy value	218.67 ± 3.21
Energy value	85.57 ± 0.00

Mean values ± standard deviation, $n = 3$.

543 Supplementary Table 1. Inhibition halo of *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus* 29213 and *Escherichia coli* 25922

544 grown on culture media pre-inoculated with different LAB strains isolated from agro-industrial waste.

			Halo			
LAB strain	**GenBank code**	**Isolation source**	***Salmonella***	***L. monocytogenes***	***S. aureus***	***E. coli***
*Weissella soli*_29-5(1)	ON763313	Carrot waste residues	+	+	+	+
*Weissella soli*_30-6(3)	ON763314	Carrot waste residues	+	+	+	+
*Weissella soli*_31-2(9B)	ON763315	Carrot waste residues	+	+	+	+
*Lactiplantibacillus pentosus*_16-6(1C)	ON763300	Coffee brush	+	+	+	+
*Leuconostoc pseudomesenteroides*_18-(1B)	ON763310	Coffee brush	++	+	+	+
*Lactobacillus pentosus*_19-(3A)	ON763312	Coffee brush	+	+	+	+
*Lactobacillus pentosus*_19-(5A)	ON763302	Coffee brush	+	+	+	+
*Leuconostoc*_66-2(4A)	ON763311	Orange waste residuals	+	+	+	+
*Levilactobacillus brevis*_68-6(1C)	ON763329	Orange waste residuals	+	+	+	+
*Lactobacillus plantarum*_69-2(3D)	ON763306	Orange waste residuals	+	+	+	+
*Lactobacillus pentosus*_70-6(1E)	ON763307	Orange waste residuals	+	+	+	+
Lactobacillus plantarum subsp. *plantarum*_70-6(13E)	ON763327	Orange waste residuals	+	+	+	+
*Lacticaseibacillus paracasei*_P2	ON763288	MFC of coffee effluent	+	+	+	+
*Lacticaseibacillus paracasei*_P4	ON763289	MFC of coffee effluent	+	+	+	+
*Lacticaseibacillus paracasei*_P6	ON763290	MFC of coffee effluent	+	+	+	+

*Lacticaseibacillus paracasei*_P8	ON763291	MFC of coffee effluent	+	+	+	+
*Lacticaseibacillus paracasei*_P9	ON763292	MFC of coffee effluent	+	+	+	+
*Lacticaseibacillus paracasei*_P10	ON763293	MFC of coffee effluent	+	+	+	+
*Lacticaseibacillus paracasei*_P13	ON763294	MFC of coffee effluent	+	+	+	+
*Limosilactobacillus fermentum*_56(6)-2F	ON763317	Trinitario cocoa	+++	+	+	+
*Limosilactobacillus fermentum*_56(6)-1F	ON763318	Trinitario cocoa	+	+	+	+
*Limosilactobacillus fermentum*_56(7)-1G	ON763319	Trinitario cocoa	+	+	+	+
*Limosilactobacillus fermentum*_57(7)-2H	ON763324	Trinitario cocoa	++	+	+	+
*Limosilactobacillus fermentum*_58(7)-1J	ON763325	Trinitario cocoa	+	+	+	++
*Limosilactobacillus fermentum*_78(6)-1A	ON763321	Trinitario cocoa	+	+	+	+
*Pediococcus acidilactici*_78(6)-3A	ON763330	Trinitario cocoa	++	++	+	+
*Limosilactobacillus fermentum*_78(6)-2A	ON763322	Trinitario cocoa	+	+	+	+
*Limosilactobacillusfermentum*_79(6)-1D	ON763323	Trinitario cocoa	+	+	+	+
*Weissellaghanensis*_80(6)-1E	ON763316	Trinitario cocoa	+	+	+	+

545 + Inhibition zone 0- 3 mm in diameter (weak), ++ inhibition zone 3- 6 mm in diameter (good), +++ inhibition zone larger than 6 mm in diameter

546 (strong). MFC=microbial fuel cells.

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548 **List of figures**

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