Probiotic potential of thermotolerant lactic acid bacteria strains isolated from cooked meat products


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Abstract

Ten thermotolerant lactic acid bacteria - Pediococcus pentosaceus (4 strains), Lactobacillus plantarum (3 strains), Enterococcus faecium (2 strains) and Aerococcus viridans (1 strain) – isolated from cooked meat products were evaluated to identify their probiotic properties, including tolerance to low pH, tolerance to taurocholic acid and bile, coaggregation and adherence to HEp-2 epithelial cells. Fifty percent of the strains were intolerant to low pH and simulated gastric juice. All strains grew in taurocholic acid and bile concentrations greater than 0.3%, indicating they are good probiotic candidates. Less than 20% of the strains coaggregated with an E. coli indicator while 30% coaggregated with a Salmonella strain. Eight of the strains exhibited good autoaggregation capacity at 24 h and all ten had a high adherence capacity for HEp-2 cells. The studied thermotolerant lactic acid bacteria are promising ingredients in the production of cooked meat products with probiotic potential.

Introduction

Probiotics are living microorganisms which provide beneficial effects for human or animal health by improving the gut microflora balance and are administered alone or incorporated into food or feed systems (Fuller, 1989). They exhibit antagonist action, produce antimicrobial substances and help to modulate immune response (Dunne et al., 1999). For a strain to qualify as a probiotic it must fulfill certain physiological characteristics, mainly survival in the gastrointestinal tract, tolerance to low pH, tolerance to bile in the form of glycocholic or taurocholic acid and sodium deoxycholate (Tomasik and Tomasik, 2003; Madigan et al., 2006). It must also have the capacity to adhere to the intestinal mucus and epithelial cells. This is important based on the two proposed mechanisms for lactic acid bacteria’s beneficial effects in the intestine: a) production of antimicrobial substances such as lactic acid and bacteriocins; and b) adherence to the mucus, coaggregation and autoaggregation to form a barrier which blocks colonization by pathogens (Ehrmann et al., 2002).

Lactic acid bacteria (LAB) are natural inhabitants of the gastrointestinal (GI) tract and are the most frequently used probiotics. Some Lactobacillus and Bifidobacterium strains have been used due to their beneficial effects on health (Lee and Salminen, 1995), although probiotic characteristics and resistance to biological barriers vary widely between species and among strains of the same species (Vinderola and Reinheimer, 2003).

The use of native lactic acid bacteria as bioprotective cultures is important for cooked meat products. Contrary to dry meat products where the starter culture become dominant changing the environment to ensure microbiological quality, cooked meat products need a thermal processing to develop texture and destroy vegetative forms in order to ensure the adequate shelf life. The lactic acid strains employed in this research have been previously studied as a biopreservative culture in cooked meat products, inoculated as free cells (Pérez-Chabela et al., 2008) or spray-dry encapsulated (Pérez-Chabela et al., 2012). These strains improved the texture of low fat sausages excreting exopolysaccharides.
In this view, the thermoduric capacity of these lactic acid strains could allow their inoculation in heat processed foods like cooked sausages, becoming dominant flora during vacuum-packed cold storage, acting like biopreservatives agents. The determination of the probiotic properties, like tolerance to gastric conditions and aggregation/coaggregation, is important since the characterization of these strains as probiotics can be useful to formulate functional cooked meat products.

Materials and Methods

Ten thermoduric lactic acid bacteria (T-LAB), previously genotypic identified by 16S rRNA analysis (Ramírez-Chavarin et al., 2010), were employed. The strains included 4 *Pediococcus pentosaceus* strains; 3 *Lactobacillus plantarum* strains; 2 *Enterococcus faecium* strains; and 1 *Aerococcus viridans* strain.

Low pH tolerance

Strain tolerance to low pH levels was evaluated following the method of Ehrmann et al. (2002). Briefly, each T-LAB strain was inoculated in MRS broth (De Man et al., 1960), and incubated at 35 ± 2°C for 24 h. They were then inoculated into fresh MRS culture medium and again incubated at 35 ± 2°C for 24 h. After incubation, they were centrifuged at 5000 x g for 10 min at 4°C, the cells washed three times with 0.1 M sodium phosphate buffer, NaH_{2}PO_{4}, and resuspended in the same volume of buffer. Each strain was diluted to 1/10 in 1X PBS and pH adjusted to 7.2 and then resuspended in 1X PBS. Each strain was inoculated into MRS medium, incubated under anaerobic conditions at 35 ± 2°C for 24 h, and survival quantified by plate counts. According to the reported by Victoria-Leon et al. (2006), abundant growth was considered above 300 CFU/mL.

Survival in simulated gastric juice

Following the method of Beumer et al. (1992), each strain was inoculated into MRS broth and incubated at 35 ± 2°C for 24 h until reaching an optical density (OD) of 1.0 at ∆=600 nm (concentration ~ 10^9 cfu / mL). After initial incubation, 10 µL of each bacterial suspension were inoculated into 10 mL simulated gastric juice (2.05 g / L NaCl; 0.60 g / L KH_{2}PO_{4}; 0.11 g / L CaCl_{2}; 0.37 g / L KCl; 0.05 g / L porcine bile; 0.1 g / L lipozyme; and 0.0133 g / L pepsin) at pH 2.0 and incubated at 35 ± 2°C for 90 min. Samples were taken at 0, 30, 60 and 90 min, diluted to 10^{-1} and 10^{-2}, 100 µL inoculated into dishes containing MRS agar and incubated at 35 ± 2°C for 48 h. Survival was quantified by plate counts on MRS agar. According to the reported by Victoria-Leon et al. (2006), abundant growth was considered above 300 CFU/mL.

Tolerance to bile salts

Strain growth in bile was evaluated according to Pedersen et al. (2004) and growth in taurocholate according to Ehrmann et al. (2002). Two types of bile salts (porcine bile extract and taurocholic acid) were used to analyze growth in both forms (i.e. conjugated as glycolic acid and taurocholic acid, and deconjugated as sodium deoxycholate). Each T-LAB strain was inoculated into MRS broth and incubated at 35 ± 2°C for 24 h. After initial incubation, 100 µL of each suspension were inoculated into 10 mL MRS broth supplemented with 0.1, 0.3, 0.5, 1.0 or 2.0% porcine bile extract and 7 mmol / L sodium taurocholate, and incubated at 35 ± 2°C for 7 h. Growth was monitored hourly and measured at 600 nm absorbance.

Coaggregation

Analysis of coaggregation was done following Yuki et al. (2000), with slight modifications. Each T-LAB strain was inoculated into MRS broth, the indicator strains *E. coli* O139:H26 and *Salmonella parera* IV O11:Z2Z23 inoculated into BT (biprotaspase) broth and all were incubated at 35 ± 2°C for 24 h. Dilutions were then done of the T-LAB suspensions until approximately 1.0 OD (λ=600 nm) (corresponding to 10^8 cfu / mL), and of the *E. coli* and *Salmonella parera* strains until 0.6 OD (λ=600 nm) (corresponding to 10^5 cfu / mL). The bacterial suspensions were then centrifuged at 7700 g for 10 min at 4°C, the cells washed three times with 0.1 M PBS and resuspended in the same volume of buffer. Optical density (OD; λ=600 nm) was adjusted to 0.5 cells for the T-LAB, *E. coli* and *Salmonella parera* strains. Mixtures were then made of each T-LAB strain with each indicator strain at a 1:1 total volume and survival quantified by plate counts. Each mixture was incubated at 35 ± 2°C for 4 h. Suspension absorbance (λ=600 nm) was measured for the mixtures, and each of the individual strains (i.e. T-LABs, *E. coli* O139:H26 and *Salmonella parera* IV O11:Z2Z23). Coaggregation percentage was calculated according to Handley et al. (1987):

\[
\text{Coaggregation} = \frac{(A_{\text{Pathogen}} + A_{\text{LAB}})}{2} - \frac{A_{\text{Mix}}}{2} \times 100
\]

Results were analyzed by a one way ANOVA and significantly difference was determined by Duncan’s mean test in SAS Software v. 8.0 (SAS Institute, Cary, USA).
Autoaggregation Analysis of autoaggregation was done following Collado et al. (2007), with slight modifications. Each T-LAB strain was inoculated into MRS broth and incubated at 35 ± 2°C for 24 h. Dilutions were then done until approximately 1.0 OD (λ=600 nm) (corresponding to 108 cfu / mL), and these suspensions centrifuged at 3200 g for 20 min at 4°C. The cells were washed three times with 1.0 M PBS, resuspended in the same volume of buffer, OD (λ=600 nm) adjusted to 1.0 and incubated at 35 ± 2°C for 2 or 24 h. Absorbance was measured after incubation and the autoaggregation percentage calculated according to Reniero et al. (1992):

\[
\text{Autoaggregation} = 1 - \left( \frac{A_{\text{time}}}{A_{\text{initial}}} \right) \times 100
\]

Where:
- \(A_{\text{time}}\) = Absorbance (λ=600 nm) at different incubation times (2 and 24 h).
- \(A_{\text{initial}}\) = Absorbance (λ=600 nm) before incubation period.

Results of incubation time and lactic acid bacteria strain were analyzed by an ANOVA and significantly difference was determined by Duncan’s mean test in SAS Software v. 8.0 (SAS Institute, Cary, USA).

HEp-2 adherence assay Adherence was evaluated following Cravioto et al. (1979) using a 75 mL cell culture flask containing 20 mL Eagle minimum essential medium (MEM) and a HEp-2 cell monolayer at 80% confluence.

Day 1. Bacterial suspension Each T-LAB strain was inoculated into tubes containing 3 mL MRS broth with 1% D-mannose (Sigma) and incubated at 35 ± 2°C for 2 or 18 h.

Day 2. Preparation of 24-well propylene plate After flaming, a tweezers was used to place plastic beads in each well. The MEM was emptied from the HEp-2 cell culture flask, and 1 mL trypsin added to rinse the flask and then discarded. Again, 1 mL trypsin was added to the flask and left for 5 min to detach the cell monolayer. After adding 29 mL MEM, 1 mL of suspension (2.5 x 10^8 cells / mL) was added to each well in the plate. After filling six wells, the cell suspension was repipetted to maintain homogeneity. The plate was incubated at 37°C in a 5% CO₂ atmosphere and 85% humidity for 24 h to produce a 90% confluence monolayer.

Day 3. Adherence assay Using a Pasteur pipette under sterile conditions, 1 mL of medium was removed from each well. The cells in the wells were washed with 1 mL 1X PBS, resuspended three times and the supernatant discarded. After washing, 1 mL 1X PBS was added, the bacterial culture centrifuged at 2000 g for 15 min and the supernatant discarded. The bacterial pellet was resuspended in 1 mL 1X PBS, and the tubes placed in an agitator to dissolve the pellet. Of this bacterial suspension, 50 μL (final concentration = 1.0 x 10^8 cfu / mL) was added to a sterile 1 mL microtube, as well as 850 μL MEM (without serum or antibiotic) and 100 μL 10% D-mannose (1% final concentration). The 1X PBS was removed from the plate wells containing the cell monolayer, 1 mL bacterial suspension added to each well and the plate incubated at 37°C in a 5% CO₂ atmosphere for 3 h. After incubation, the wells were washed in triplicate with 1 mL 1X PBS. Concentrated methanol (500 μL) was added for 1 min to fix, 1 mL Giemsa dye added for 20 min and the wells washed in triplicate with 1 mL deionized water to eliminate excess dye. Cells were dehydrated by picking up each bead and running it for 30 s each through a series of acetone, acetone 50 / xylene 50 and xylol 100. The beads were immediately mounted in Canada balsam on a slide, a drop of resin placed over each bead and allowed to dry for 24 h. Once dry the slides were viewed with a microscope and photographed with an integrated HBO 50 camera. Adherence was indicated to be positive if more than ten T-LAB adhered per HEp-2 epithelial cell and degree of adherence was noted. This analysis was done in triplicate.

Results and Discussion Low pH tolerance Tolerance to low pH levels and bile salts is vital for bacteria to survive and grow in the GI tract, making these the main requirements for bacteria to be considered probiotic (Havenaar et al., 1992). Among the ten studied T-LAB, all exhibited good growth after 4 h incubation at pH 4 and 5 since optimum pH for LAB is between 4.5 and 6.5 (Prescott et al., 1999) (Table 1). At pH values of 1, 2 and 3, Enterococcus faecium (10a) exhibited only slight growth after 1 h and then died. Enterococcus faecium (18) had slight growth at pH 0.5, 1, 2 and 3 after 1, 2 and 4 h incubation. In contrast, Lactobacillus plantarum (17) exhibited good growth values at different pH values for 1 h but did not grow further at 2 and 4 h. Bacteria are generally sensitive to the stomach’s low pH values (Conway et al., 1987), however, some LAB can survive and grow at relatively low pH because they have a system that simultaneously transports lactic acid and protons to the cell’s exterior.
In a study similar to the present study, *Lactobacillus* GG survived pH 3 for 4 h (Goldin *et al*., 1992), while in another study *L. casei* 212.3 and *Lactobacillus* GG exhibited good growth at pH 2.3 for 3 h (Charteris *et al*., 1998). In the latter study, the authors classified the strains as intrinsically tolerant, meaning that when ingested under fasting conditions they were intrinsically resistant to transit through the human GI tract, meaning a large amount of microorganisms could therefore arrive at the upper intestine. Under the same conditions, *L. casei* F19 decreased from $10^9$ to $10^3$ cfu / mL, causing the authors to classify it as intrinsically sensitive, that is, intolerant of GI tract conditions (Charteris *et al*., 1998). In another study, 99 *Lactobacillus casei* strains were shown to resist pH 3 for 3 h, but the strains *Lactobacillus casei* NCDC 17 and *Lactobacillus casei* C1 were able to resist pH 2 for 3 h (Mishra and Prasad, 2005).

Overall, the ten T-LAB strains studied here were comparatively intolerant of low pH conditions. Nonetheless, systems such as microencapsulation can improve probiotic survival when exposed to acid conditions, bile salts and thermal treatments (Ding & Shah, 2007).

**Table 1. Thermotolerant lactic acid bacteria tolerance to low pH**

<table>
<thead>
<tr>
<th>pH</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>A. viridans (21)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. faecium (10a)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E. faecium (18)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L. plantarum (15c)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L. plantarum (17)</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>P. pentosaceus (11)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. pentosaceus (12)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. pentosaceus (15a)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. pentosaceus (15L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. pentosaceus (22)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+++ Abundant growth, ++ Moderate growth, + Poor growth, - No growth

**Table 2. Thermotolerant lactic acid bacteria tolerance to different bile concentrations**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Bile concentrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
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<tr>
<td>A. viridans (21)</td>
<td>++</td>
</tr>
<tr>
<td>E. faecium (10a)</td>
<td>++</td>
</tr>
<tr>
<td>E. faecium (18)</td>
<td>++</td>
</tr>
<tr>
<td>L. plantarum (15c)</td>
<td>++</td>
</tr>
<tr>
<td>L. plantarum (17)</td>
<td>++</td>
</tr>
<tr>
<td>P. pentosaceus (11)</td>
<td>++</td>
</tr>
<tr>
<td>P. pentosaceus (12)</td>
<td>++</td>
</tr>
<tr>
<td>P. pentosaceus (15a)</td>
<td>++</td>
</tr>
<tr>
<td>P. pentosaceus (15L)</td>
<td>++</td>
</tr>
<tr>
<td>P. pentosaceus (22)</td>
<td>++</td>
</tr>
</tbody>
</table>

++ Abundant growth, + Poor growth

In a study similar to the present study, *Lactobacillus* GG survived pH 3 for 4 h (Goldin *et al*., 1992), while in another study *L. casei* 212.3 and *Lactobacillus* GG exhibited good growth at pH 2.3 for 3 h (Charteris *et al*., 1998). In the latter study, the authors classified the strains as intrinsically tolerant, meaning that when ingested under fasting conditions they were intrinsically resistant to transit through the human GI tract, meaning a large amount of microorganisms could therefore arrive at the upper intestine. Under the same conditions, *L. casei* F19 decreased from $10^9$ to $10^3$ cfu / mL, causing the authors to classify it as intrinsically sensitive, that is, intolerant of GI tract conditions (Charteris *et al*., 1998). In another study, 99 *Lactobacillus casei* strains were shown to resist pH 3 for 3 h, but the strains *Lactobacillus casei* NCDC 17 and *Lactobacillus casei* C1 were able to resist pH 2 for 3 h (Mishra and Prasad, 2005).

Overall, the ten T-LAB strains studied here were comparatively intolerant of low pH conditions. Nonetheless, systems such as microencapsulation can improve probiotic survival when exposed to acid conditions, bile salts and thermal treatments (Ding & Shah, 2007).

**Survival in simulated gastric juice**

In the simulated gastric juice survival trial, three *Pediococcus pentosaceus* strains (11, 15L and 15a), *Aerococcus viridans* (21) and *Enterococcus faecium* (18) remained viable during the 90 min incubation (Figure 1a). *Pediococcus pentosaceus* (12) and...
two *Lactobacillus plantarum* strains (15c and 17) exhibited a reduction of one logarithmic cycle during the first 30 min. At 60 min incubation, *Pediococcus pentosaceus* (22) experienced a reduction of two logarithmic cycles and *Enterococcus faecium* (10a) one of 1.5 logarithmic cycles (Figure 1b).

Bacteria intended to exercise beneficial effects in the intestines must survive transit through the stomach’s acid environment. The present results demonstrate that 50% of the studied T-LAB strains could survive these conditions for 90 min, long enough to reach their action site in the intestine (Fernandez et al., 2003).

**Bile tolerance**

Some *Lactobacillus* strains are capable of surviving high bile concentrations and can therefore adapt to GI tract conditions (Gilliland et al., 1984). Of the ten studied T-LAB strains, five grew effectively at all five tested bile concentrations (0.1, 0.3, 0.5, 1.0 and 2.0%) (Table 2): *Pediococcus pentosaceus* (12); *Pediococcus pentosaceus* (15L); *Pediococcus pentosaceus* (22); *Enterococcus faecium* (10a); and *Pediococcus pentosaceus* (15a). The remaining five strains exhibited abundant growth at the 0.5% concentration, but lower growth rates at the 1 and 2% concentrations: *Pediococcus pentosaceus* (11), *Aerococcus viridans* (21), *Lactobacillus plantarum* (15c), *Lactobacillus plantarum* (17) and *Enterococcus faecium* (18). These results coincide with an evaluation of *Lactobacillus* strains isolated from the chicken GI tract in which strains resistant to acid pH and bile salts were selected (Rondón et al., 2008): twenty strains were identified with probiotic activity, six of which had the best tolerance to low pH and bile salts. This is similar to an earlier study in which twelve *Lactobacillus* strains isolated from the chicken GI tract were reported to survive low pH and a 0.3% bile concentration (Jin et al., 1998). This bile salt concentration is critical for detecting resistant bacterial strains (Gilliland et al., 1984), meaning all ten T-LAB tested in the present study are candidates for use as probiotics since all grew at a bile concentration greater than 0.3%.

**Taurocholic acid tolerance**

Bile is a steroid produced by the liver and secreted through the bile duct in the form of bile salts. These salts can occur as conjugates between cholic acid and the amino acids glycine or taurine (forming glycholic or taurocholic acid), or as deconjugates such as sodium deoxycholate (Madigan et al., 2006). The five *Pediococcus* sp. tested in the present study grew well in 7 mM taurocholic acid compared to the control (Figure 2a), as did the strains *Aerococcus viridans* (21), *Lactobacillus plantarum* (17), *Enterococcus faecium* (10a) (Figure 2b). The exception was *Lactobacillus plantarum* (15c), which did not survive this hostile medium. This coincides with previous reports of some *Lactobacillus* strains not surviving conjugated bile salts, probably due to an absence of the enzymes needed to hydrolyze them, but achieving growth in the presence of deconjugated bile salts (Suskovic et al., 1997). Similar results were reported in an *in vitro* study of *Lactobacillus reuteri* strains with hydrolytic activity for bile salts in which the bile salts had no negative effect on strain growth probably because the deconjugated salts were adsorbed onto the lactobacillus cell surface, thus diminishing their bioavailability (De Boever et al., 2000). In another study, most probiotic bacteria were reported to grow in MRS medium supplemented with more than 0.5% conjugated bile salts (Noriega et al., 2006).

**Coaggregation**

Aggregation between cells of the same strain (autoaggregation) or different species (coaggregation) is an example of bacterial interaction (Kmet et al., 1995). Coaggregation may improve pathogen
Table 3 Thermorolerant lactic acid bacteria coaggregation capacity with other strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>%Coaggregation with E. coli O139:H26</th>
<th>%Coaggregation with Salmonella parera IV O11:Z4Z23</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. viridans (21)</td>
<td>3.34 ± 2</td>
<td>5.33 ± 2</td>
</tr>
<tr>
<td>E. faecium (10a)</td>
<td>5.06 ± 2</td>
<td>16.11 ± 2</td>
</tr>
<tr>
<td>E. faecium (18)</td>
<td>23.56 ± 2</td>
<td>32.71 ± 2</td>
</tr>
<tr>
<td>L. plantarum (15c)</td>
<td>6.31 ± 2</td>
<td>8.81 ± 2</td>
</tr>
<tr>
<td>L. plantarum (17)</td>
<td>2.49 ± 2</td>
<td>0.48 ± 2</td>
</tr>
<tr>
<td>P. pentosaceus (11)</td>
<td>5.72 ± 2</td>
<td>4.87 ± 2</td>
</tr>
<tr>
<td>P. pentosaceus (12)</td>
<td>26.33 ± 2</td>
<td>2.13 ± 2</td>
</tr>
<tr>
<td>P. pentosaceus (15a)</td>
<td>5.97 ± 2</td>
<td>6.71 ± 2</td>
</tr>
<tr>
<td>P. pentosaceus (15L)</td>
<td>12.55 ± 2</td>
<td>17.29 ± 2</td>
</tr>
<tr>
<td>P. pentosaceus (22)</td>
<td>11.04 ± 2</td>
<td>1.80 ± 2</td>
</tr>
</tbody>
</table>

Means with same letter in same row are not significantly different (P<0.05) for incubation time.

*Means with same letter in same column are not significantly different (P<0.05) for lactic acid bacteria strain.

Table 4. Thermorolerant lactic acid bacteria autoaggregation capacity (%) after 2 and 24 h incubation time

<table>
<thead>
<tr>
<th>Strain</th>
<th>Incubation time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>A. viridans (21)</td>
<td>20.24 ± 9.9</td>
</tr>
<tr>
<td>E. faecium (10a)</td>
<td>7.39 ± 8.9</td>
</tr>
<tr>
<td>E. faecium (18)</td>
<td>2.90 ± 8.9</td>
</tr>
<tr>
<td>L. plantarum (15c)</td>
<td>1.24 ± 8.9</td>
</tr>
<tr>
<td>L. plantarum (17)</td>
<td>15.30 ± 7.9</td>
</tr>
<tr>
<td>P. pentosaceus (11)</td>
<td>18.96 ± 8.9</td>
</tr>
<tr>
<td>P. pentosaceus (12)</td>
<td>3.59 ± 8.9</td>
</tr>
<tr>
<td>P. pentosaceus (15a)</td>
<td>23.25 ± 8.9</td>
</tr>
<tr>
<td>P. pentosaceus (15L)</td>
<td>4.26 ± 8.9</td>
</tr>
<tr>
<td>P. pentosaceus (22)</td>
<td>17.74 ± 8.9</td>
</tr>
</tbody>
</table>

Means with same letter in same row are not significantly different (P<0.05) for incubation time.

*Means with same letter in same column are not significantly different (P<0.05) for thermorolerant lactic acid bacteria strain.

elaboration (via dragging) and/or destruction in the GI tract in that it may optimize the effect of antibacterial substances emitted by probiotic microorganisms against the pathogenic bacteria with which they coaggregate (Charteris et al., 1998). All the T-LAB strains tested in the present study coaggregated with the E. coli O139:H26 and Salmonella parera IV O11:Z4Z23 as indicator strains (Table 3). E. faecium (18) obtained the significantly (P<0.05) higher coaggregation percent values for both E. coli and Salmonella parera strains. Although P. pentosaceus (12) obtained the significantly (P<0.05) higher values with E. coli, its coaggregation with S. parera was significantly (P<0.05) lower than the rest of the T-LAB. Both P. pentosaceus (15L) and (15a) obtained significantly (P<0.05) acceptable coaggregation percent as well.

Coaggregation may be a vital factor in establishing and maintaining microflora with the aim of preventing gastrointestinal and urogenital bacterial infections. The presence of Lactobacillus in the urogenital epithelium and their ability to interact with uropathogens may be an important host defense mechanism against infections (Reid et al., 1988). In the present study, 20% of the T-LAB stains interacted with E. coli and 30% interacted with Salmonella, indicating they could function to eliminate pathogenic bacteria from the GI tract.

Autoaggregation

This phenomenon is very important in a number of ecological niches, particularly in the human gut, the most common intended action site for probiotics (Jankovic et al., 2003). As expected, all the strains showed a significantly (P<0.05) higher aggregation capacity after 24 h of incubation time (Table 4). The thermorolerant lactic acid strains with significantly (P<0.05) higher autoaggregation percents were A. viridans (21), L. plantarum (17) and P. pentosaceus (11). The significantly (P<0.05) lower autoaggregation was for E. faecium (18), L. plantarum (12), L. plantarum (15c) and P. pentosaceus (15a). Their high autoaggregation capacities suggest these strains could be very useful in forming biofilms and/or in GI tract colonization, which are ways of forming a barrier against colonization by pathogenic microorganisms (Schachtsiek et al., 2004; Schellenberg et al., 2006). This capacity may be the result of their modification of the microenvironment surrounding pathogens and/or the production of inhibitory substances by the T-LAB (Pérez et al., 1998; Del Re et al., 2000).

In a study of cell surface adherence and aggregative capacity in Lactobacillus plantarum and Enterococcus faecium strains. Collado et al. (2007) reported that autoaggregative capacity is correlated to adherence, which is a prerequisite for GI tract colonization and infection by pathogens. Coaggregation, in contrast, is linked to the ability to interact closely with pathogens. These authors concluded that autoaggregative capacity, together with cell surface hydrophobicity and the ability to coaggregate with pathogenic strains can be used for preliminary selection and identification of probiotic bacteria with potential applications in human and animal systems.

HEP-2 cell adherence

A principal criterion for probiotic strain selection is the capacity to adhere to the intestinal surface since adherence to the intestinal mucus is required for colonization and antagonistic activity against enteropathogens (Granato et al., 1999). Upon arriving in the intestine, a probiotic strain must fix itself to the tips of the microvilli and then adhere itself to the mucus to avoid being swept off by peristalsis (Fernández et al., 2003). The intestinal mucus is a classic model for testing in vitro adherence since different receptors can be located in the small and large intestine mucus using the specific adherence
properties of a number of beneficial and pathogenic bacteria (Li et al., 2008).

All ten of the studied T-LAB strains met the minimum adherence criterion of ten bacteria adhered per HEp-2 epithelial cell (derived from human pharynx cancer cells) (Cravioto et al., 1979; Ehrmann et al., 2002). In addition to this overall positive adherence, over 40% of the cells exhibited levels indicating strong adherence (Figs. 3a-f, Figs. 4a-f). The one exception was the comparatively lower adherence of *Pediococcus pentosaceus* (22) (Figure 3-f), although it still met the criterion for positive adherence (Ehrmann et al., 2002).

In contrast to previous adherence studies using *Lactobacillus* strains, which employed Caco-2 and HT-29 intestinal cell lines, the HEp-2 cell line was used in the present study. The results are still comparable to those produced using the intestinal cell lines since the HEp-2 cell line is a model system used to test the adherence of enterotoxic *E. coli* strains which cause diarrheic disorders in humans and animals. Its surface has pili which adhere to intestinal epithelial cells and colonize the small intestine (Smith & Longgood, 1971). This model is therefore valid for evaluating the adherence and colonization of T-LAB in the GI tract.

The adherence observed here for the ten studied T-LAB strains is consistent with previous reports of *Lactobacillus* adhering to human intestinal cells via a mechanism involving different combinations of carbohydrates and proteins in the bacteria cell’s surface (Chauvière et al., 1992). The present results also contained patterns similar to those reported in other studies in which four adherence patterns are described: aggregative adherence; diffuse adherence; localized adherence; and localized adherence-like (Nataro et al., 1987; Vidal et al., 2007). Eight of the studied T-LAB strains exhibited a diffuse adherence pattern: *Pediococcus pentosaceus* (11); *Pediococcus pentosaceus* (15 L); *Aerococcus viridans* (21); *Enterococcus faecium* (10a); *Pediococcus pentosaceus* (15a); *Lactobacillus plantarum* (15c); *Lactobacillus plantarum* (17); and *Enterococcus faecium* (18). *Pediococcus pentosaceus* (12) exhibited a localized adherence-like pattern.

A number of studies have been done on adherence in potential probiotic LAB, using both the HEp-2 and Caco-2 cell lines. Ehrmann et al. (2002) evaluated 112 LAB strains from duck using in vitro methods to determine autoaggregation, coaggregation, surface hydrophobicity and adherence to HEp-2 cells, among other characteristics. They observed significant autoaggregation and coaggregation, as well as strong adherence to HEp-2 cells, and concluded that most of the tested *Lactobacillus* strains were potential probiotics. Using the Caco-2 cell line as an in vitro model, Tuomola and Salminen (1998) compared adherence among twelve *Lactobacillus* strains, observing the highest adherence in *L. casei* (14.4%) and the lowest in *L. casei* rhamnosus (2.6%). They concluded that these potential probiotic *Lactobacillus* strains had variable ability to adhere to intestinal cells, possibly attributed to strain source and/or surface properties. In another study of adherence capacity in eleven *Lactobacillus* strains, Pennacchia et al. (2006) used an in vitro Caco-2 model to find that all eleven strains had good adherence capacity to the Caco-2 cell monolayer with eight strains from the group *Lactobacillus plantarum* exhibiting the highest number of adhered cells. Delgado et al.
(2008) analyzed the probiotic characteristics of 187 Bifidobacterium strains isolated from healthy adult mucus and feces, including an in vitro adherence assay using the Caco-2 cell line. All the tested strains adhered to the epithelial cells, and they proposed the strains Bifidobacterium catenulatum, Bifidobacterium longum and Bifidobacterium pseudocatenulatum as new probiotic candidates. Based on the present results and compared to the above studies, the ten studied T-LAB strains had good adherence capacity to HEP-2 cells and therefore could adhere to intestinal cells.

Conclusions

Most of the studied T-LAB strains presented an adequate resistant to low pH and bile salt concentrations. These characteristics are important requisites for a probiotic strain, and also exhibited high adherence capacity to HEP-2 epithelial cells. The inherent thermostolerant capacity (probably resulting from their isolation from cooked emulsified meat products) plus the demonstrated probiotic properties made these strains a viable bioprotective culture that can be inoculated in cooked meat products before thermal processing, in order to ensure their prevalence as dominant probiotic flora during and before shelf-life.

References


some probiotic and dairy Lactobacillus strains to Caco-2 cell cultures. International Journal of Food Microbiology, 41, 45-51.


