Food Control 37 (2014) 251–256

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18s

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ARTICLE INFO

Article history:
Received 13 June 2013
Received in revised form
13 September 2013
Accepted 23 September 2013

Keywords:
Enterococcus durans
Probiotic
Antimicrobial activity
Antioxidant properties

ABSTRACT

The probiotic potential, antimicrobial and antioxidant properties of *Enterococcus durans* LAB18s, a strain capable of selenium bioaccumulation, was investigated. *E. durans* LAB18s showed resistance to acid conditions, showing ability to survive in the presence of simulated gastric juice at pH 3. This bacterium also survived in the presence of simulated intestinal juice with or without bile salts, and did not show hemolytic activity. The antimicrobial activity of culture supernatant and intracellular extract of *E. durans* LAB18s was tested against different pathogenic microorganisms, namely *Listeria monocytogenes*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Corynebacterium fimi*. *E. durans* LAB18s exhibited a broad inhibitory spectrum, except to *B. cereus*, *S. aureus* and *S.* Enteritidis when the culture supernatant was used, and to *S.* Typhimurium when the intracellular extract was tested. The antioxidant activity of culture supernatant and intracellular extract of *E. durans* LAB18s was analyzed by $ABTS^{\bullet+}$ and DPPH methods, and only culture supernatant presented ability to scavenge both radicals. Both culture supernatant and intracellular extract showed high antioxidant activity when analyzed by TBARS method. *E. durans* LAB18s could be useful as a source of dietary selenium supplementation.

1. Introduction

Enterococci are lactic acid bacteria (LAB) that have an important role in the environment, food and clinical microbiology. Furthermore, they are regular habitants of the gastrointestinal tract of both humans and animals (Bhardwaj, Kaur, Gupta, Vij, & Malik, 2011). Enterococci also occur naturally into, or are deliberately added to fermented foods, contributing to the sensory properties. Moreover, several strains of the genus *Enterococcus* have also been used as probiotics, which may improve the microbial balance of the intestine or can be used in the treatment of gastroenteritis in humans and animals (Giraffa, 2003; Foulquié Moreno, Sarantinopoulos, Tsakalidou, & De Vuyst, 2006).

The microbiota that inhabits the human intestinal tract is part of an extremely complex ecological system. These microorganisms interact not only with other microorganisms, but also with their host. Among intestinal microbiota, LAB play significant role in the gut ecosystem (Lin & Chang, 2000). The gram positive LAB has been well known for thousands of years for their important function in the food industry due to their fermentative capacities. In current years, the role of these bacteria in health and functionality of human and livestock animal intestine have been well emphasized, mainly because of their ability to growth in low pH and to produce antimicrobial agents (Foulquié Moreno et al., 2006). Likewise, the antioxidant potential of LAB has been suggested by some researchers (Lobo, Patil, Phatak, & Chandra, 2010; Ou et al., 2009).

Promising probiotic strains include members of the genera *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* (Buntin, Suphitchaya, & Tipparat, 2008). Many LAB, including the genus *Enterococcus*, are proved with probiotic functions, which are beneficial to the host when ingested in sufficient quantities. The colonization of the gut by probiotic bacteria prevents growth of harmful bacteria by competition exclusion and by the production of organic acids and antimicrobial compounds. The acid and bile tolerance are two fundamental properties that indicates the ability

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http://dx.doi.org/10.1016/j.foodcont.2013.09.055

of a probiotic microorganism to survive through the upper gastrointestinal tract (Erkkila & Petaja, 2000; Hyronimus, Marrec, Hadj, & Deschamps, 2000). The viability and activity of probiotic bacteria are important for survival in food during shelf life and transition through the acidic conditions of the stomach. To be potentially probiotics, bacteria must also be resistant to degradation by hydrolytic enzymes and bile salts in the small intestine (Belma & Gulcin, 2009).

Addition of probiotics to feed is an interesting alternative to the use of antibiotics, which have created great public concerns due to emergence of antimicrobial resistance (Patterson & Burkholder, 2003). Also, selenium is one of the essential nutritional elements whose function consists in the protection of cells and tissues from oxidation damage, and the use of microbial biomass enriched with this mineral can be considered as effective organic selenium supplement (Ren, Zhao, Wang, & Huang, 2011; Svoboda, Fajt, Banoch, Drábek, & Saláková, 2011; Zhang et al., 2009). *Enterococcus durans* LAB18s is capable to growth in selenium enriched medium, accumulating this mineral in the biomass. Thus, the aim of this study was to characterize the probiotic potential of the *E. durans* LAB18s through acid and bile salts resistance, survival in simulated gastrointestinal tract conditions, and also to evaluate its antimicrobial and antioxidant properties.

2. Materials and methods

2.1. Microorganism and growth conditions

E. durans strain LAB18s isolated from Minas Frescal cheese (typical Brazilian soft cheese), belonging to the collection of the Laboratory of Applied Microbiology and Biochemistry (Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil), was used in this study. The strain was maintained as frozen stock cultures in Brain Heart Infusion (BHI, Oxoid) containing 20% (v/v) glycerol. The bacterium was grown in BHI broth at 37 °C. To confirm selenium bioaccumulation, the strain was grown in BHI broth containing 15 mg/ml $NaSeO_3$ and the selenium content in the biomass was determined by optical emission spectrometry with inductively coupled plasma (ICP-OES) (Altundag, & Mustafa, 2011).

2.2. Acid tolerance

The resistance under acid conditions was carried out according to Erkkila & Petaja (2000) with some modifications. *E. durans* LAB18s cells were grown in BHI without shaking at 37 °C for 24 h. Then, the culture was standardized at an optical density (OD_{600}) = 1.0 ± 0.05. One milliliter of standardized culture was added into tubes containing 10 ml of sterile BHI broth with the following pH values: 2.0, 3.0, 4.0 and 7.0 (adjusted with HCl), in which pH 7.0 was used as a control. Viable cell counts were determined after exposure to acidic condition for 0, 1, 2, 3 and 4 h at 37 °C. The experiment was performed in triplicate. Survival cell counts were expressed as log values of colony-forming units per ml (CFU/ml). The survival percentage was calculated as follows: % survival = final (CFU/ml)/control (CFU/ml) × 100.

2.3. Resistance to bile salts

After *E. durans* LAB18s was grown in BHI broth, bacteria were harvested by centrifugation (10,000 × *g* for 10 min at 4 °C) and the assessment of bacterial resistance to bile salts was performed in 10 ml sterile BHI supplemented with a mixture of sodium cholate and sodium deoxycholate (Sigma) in a ratio of 1:1, achieving final concentrations of 0.1, 0.25, 0.5, 1.0 and 1.5% (w/v). Total viable counts were determined after exposure to bile salts at 0, 1, 2, 3 and 4 h of incubation, by pour plate method with BHA after serial dilutions of the sample and incubation at 37 °C for 24 h. Values were expressed as log CFU/ml (Perelmuter, Fraga, & Zunino, 2008).

2.4. Survival in simulated gastrointestinal tract

Survival in simulated gastrointestinal tract was performed according to Huang and Adams (2004). After 24 h of incubation in BHI medium, bacterial cells were harvested by centrifugation (10,000 × *g* for 10 min at 4 °C), washed three times with 0.1 M phosphate buffered saline (PBS) (pH 7.2) and suspended in 0.5% NaCl solution. Then, a 0.2 ml aliquot of bacterial suspension was inoculated into 1.0 ml of simulated gastric or intestinal juices and incubated at 37 °C for 4 h. Survival cell counts were determined at initial time (0 h) and 1, 2, 3 and 4 h for the gastric tolerance and intestinal tolerance. Values were expressed as log CFU/ml.

Simulated gastric juice was prepared fresh daily containing 3 mg of pepsin (Sigma), 1 ml of NaCl solution (0.5%) and acidified with HCl to pH 2.0 or 3.0. Simulated intestinal juice was consisted of 1 mg of pancreatin (Merck), 1 ml of NaCl solution (0.5%) and adjusted to pH 8.0, with or without 1.5% of bile salts (1:1 mixture of sodium cholate and sodium deoxycholate). Both solutions were sterilized by filtration through 0.22 μm membranes (Millipore, Bedford, USA).

2.5. Hemolytic activity

The strain was tested for hemolytic activity using blood agar (7% v/v sheep blood) for 48 h incubation at 37 °C (Foulquié Moreno et al., 2003). Strains that produced green-hued zones around the colonies (α-hemolysis) or did not produce any effect on the blood plates (γ-hemolysis) were considered non hemolytic. Strains displaying blood lyses zones around the colonies were classified as hemolytic (β-hemolysis).

2.6. Preparation culture supernatant and intracellular extract

E. durans LAB18s was inoculated in 10 ml BHI and incubated at 37 °C for 24 h. Aliquots of the culture were transferred to 2 ml polypropylene tubes, and centrifuged at 10,000 × *g* for 15 min at 4 °C. The resulting supernatant was neutralized (pH 7.0) with 1 M NaOH and heated at 95 °C for 5 min (Bromberg, Moreno, Delboni, & Cintra, 2006). This culture supernatant was used to evaluate the antimicrobial and antioxidant activity.

For preparation of intracellular extracts, the cell pellet was washed twice with ultrapure water and suspended in ultrapure water, followed by ultrasonic disruption (Unique USC 700). The sonication was performed at 40 kHz during 15 min, with five intervals of 1 min in an ice bath. Cellular debris was removed by centrifugation at 10,000 × *g* for 15 min at 4 °C. The resulting supernatant was used as cell extract to evaluate the antimicrobial and antioxidant activity.

2.7. Antimicrobial activity

The indicator microorganisms used to evaluate antimicrobial activity were: *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 8739, *Bacillus cereus* ATCC 9634, *Staphylococcus aureus* ATCC 1901, *Salmonella* Typhimurium ATCC 14078, *Salmonella* Enteritidis ATCC 13076, *Pseudomonas aeruginosa* (isolated from food), *Aeromonas hydrophila* 00318 IOC/FDA 110-36 and *Corynebacterium fimi* NCTC 7547. They were suspended in 0.85% NaCl solution standardized to OD_{600} of 0.150 in spectrophotometer, which corresponded to a 0.5 McFarland turbidity standard solution. One aliquot of 20 μl of culture supernatant of *E. durans* LAB18s was applied on

sterilized cellulose discs (5 mm) onto BHA plates previously inoculated with a swab soaked in a culture of each indicator bacteria. The plates were incubated at 37 °C and inhibition zones were measured after 24 h. The same procedure was performed to evaluate the antimicrobial activity of intracellular extract. The diameter of inhibition zones was measured using a caliper and halos ≥7 mm were considered inhibitory (Bromberg et al., 2006). The experiment was performed in triplicate.

2.8. Thiobarbituric acid reactive substances (TBARS)

The reaction to thiobarbituric acid was performed according to the methodology of Ohkawa, Ohishi, and Yagi (1979). Test tubes containing ultrapure water and extra virgin olive oil were subjected to oxidation with 100 μM ferrous sulfate and incubated in a water bath at 80 °C, for 10 min. Thereafter, to each tube was added the samples (culture supernatant or intracellular extract of the bacteria), 81 mg/ml of sodium dodecyl sulfate (SDS) buffered with acetic acid at pH 3.44, and 6 mg/ml thiobarbituric acid (TBA). The reaction mixture was further incubated in a water bath at 100 °C for 1 h. Each sample tested had a blank to either the culture supernatant or intracellular extract, and a standard control for all comparisons. The reaction products were determined by measurement of absorbance at $\lambda = 532$ nm with a spectrophotometer. The concentration of TBARS was calculated using a standard curve developed with known concentrations of 1,1,3,3-tetramethoxypropane, and results were expressed as nmol of malonaldehyde (MDA)/ml of sample. The experiment was performed in triplicate.

2.9. Antioxidant capacity using $ABTS^{\bullet+}$ method

The antioxidant activity was determined using $ABTS^{\bullet+}$ (2,2 azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation method (Re et al., 1999) with some modifications described by Rossini, Noreña, Olivera, and Brandelli (2009). $ABTS^{\bullet+}$ was dissolved in water (7 mM). ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in dark at room temperature for 16 h before use. The stock solution was used for a maximum of 3 days. Before use, $ABTS^{\bullet+}$ solution was diluted with ethanol, to an absorbance of 0.700 ± 0.020 at 734 nm. Samples were diluted with ethanol to obtain inhibition between 20 and 95% of the blank absorbance. Ascorbic acid was used as the standard in the range 0–9 μg/ml. After addition of 10 μl of sample (or standards) in 1.0 ml of $ABTS^{\bullet+}$ solution, the absorbance was read at 30 s interval for 5 min. Likewise, a same proportion (10 μl) of culture medium or ultrapure water were used as controls. All determinations were carried out at least three times. The percentage inhibition of absorbance at 734 nm was calculated using ascorbic acid standard curve (Rossini et al., 2009).

2.10. Scavenging ability on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals

The DPPH method (Brand-Williams, Cuvelier, & Berset, 1995) was based on the capture of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical by antioxidants, producing a decrease in absorbance at 515 nm. The DPPH was used at a concentration of 60 μM in methyl alcohol. The solution was homogenized, transferred to a dark glass bottle and used only in the day of analysis. In the dark, aliquots of 0.1 ml of sample (culture supernatant or intracellular extract) were transferred to test tubes containing 3.9 ml of DPPH radical solution and homogenized by shaking. A solution prepared from 50% methyl alcohol, 70% acetone and water, mixed with 3.9 ml of DPPH solution was used as control. Methyl alcohol was used as a blank. The standard curve was prepared with DPPH in the range between 0 and 60 μM. The results were expressed as EC_{50} (μg/ml), which is the minimum antioxidant concentration required to reduce 50% of the initial DPPH reaction from the time the extract reached stability.

3. Results

3.1. Tolerance to pH, bile salts and gastrointestinal juices

The isolate exhibited high tolerance to acidic conditions (Table 1). The resistance of the isolate was observed after exposition to acidified media, except for pH 2, where the presence of colonies was only observed in the initial time (>7 log CFU/ml). In the other treatments, pH 3 and pH 4, no significant differences were observed when the samples were compared to the control (pH 7) during the incubation time, ranging between 0 and 4 h.

The tolerance of *E. durans* LAB18s in the presence of different concentrations of bile salts was analyzed (Fig. 1). The results showed that *E. durans* LAB18s was able to survive at all bile salt concentrations tested (up to 1.5%) to give an exponential growth from the inoculation (0 h) until 4 h of incubation. There were no significant differences between control (BHI, pH 7, without the addition of bile salts) and different treatments with bile salts.

The ability of *E. durans* LAB18s to survive in the presence of simulated gastric juice was tested by incubation for 4 h at 37 °C (Fig. 2). It was observed that simulated gastric juice at pH 3 caused no significant differences in cell viability when compared to the control in any of the evaluated times. When the resistance in pH 2 was analyzed, it was observed that cell viability was only apparent at time 0 (6.53 log CFU/ml), and after 1 h, cell counts were below the detection limit. This result indicates that the isolate *E. durans* LAB18s was not resistant to simulated gastric juice at pH 2.

Fig. 3 shows the survival of *E. durans* LAB18s in the presence of simulated intestinal juice. Similar values of viable cell counts of *E. durans* LAB18s were observed during 4 h incubation in simulated juices and control. After 4 h the values for pH 8.0 with pancreatin, pH 8.0 with pancreatin and 1.5% bile salts, and control were 8.34, 8.56 and 8.75 log CFU/ml, respectively, demonstrating that *E. durans* LAB18s presents resistance when exposed to the simulated intestinal juice.

3.2. Hemolytic activity

The isolate *E. durans* LAB18s did not exhibited any effect (γ-hemolysis); green area (α-hemolysis), and/or inhibition zone (β-hemolysis) after 48 h incubation in blood agar plates (data not shown).

3.3. Antimicrobial activity

E. durans LAB18s exhibited antimicrobial activity against different indicator microorganisms, including *L. monocytogenes*,

Table 1
Acid tolerance of *E. durans* LAB18s after exposure to acidic conditions (pH 2, 3 and 4) during 4 h of incubation at 37 °C with shaking.

Time (h)	Log CFU/ml[a]			
	Control (pH 7)	pH 2	pH 3	pH 4
0	7.49 ± 0.01	7.18 ± 0.02	7.35 ± 0.01	7.40 ± 0.01
1	7.97 ± 0.02	0.00 ± 0.00	7.33 ± 0.01	7.33 ± 0.02
2	8.33 ± 0.01	0.00 ± 0.00	7.06 ± 0.04	7.33 ± 0.02
3	8.69 ± 0.01	0.00 ± 0.00	6.97 ± 0.04	7.31 ± 0.02
4	8.81 ± 0.02	0.00 ± 0.00	6.91 ± 0.03	7.32 ± 0.02

[a] Values represent the mean ± s.e.m. of three independent experiments.

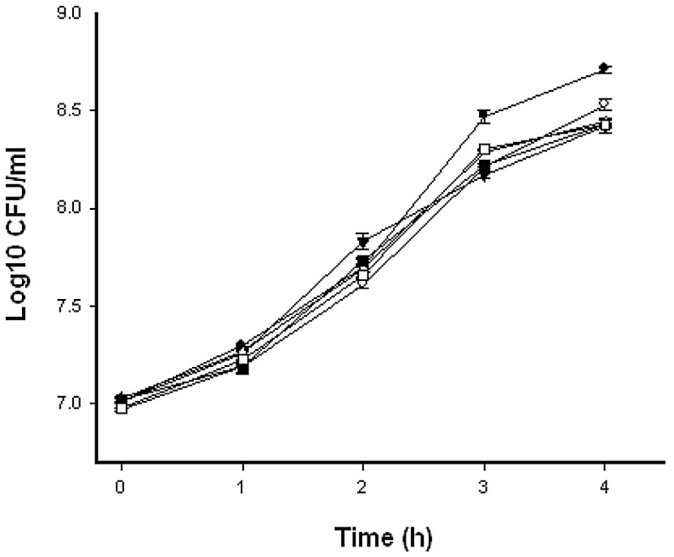

Fig. 1. Tolerance of the *E. durans* LAB18s to bile salts concentration, containing 0.1% of bile salts (○); 0.25% (▼); 0.5% (△); 1.0% (■); and 1.5% (□) after 4 h of incubation at 37 °C without shaking. The pH 7.0 without bile salts was used as a control (●). Each point represents the mean ± s.e.m. of three independent experiments.

E. coli, *B. cereus*, *S. aureus*, *S.* Typhimurium, *S.* Enteritidis, *P. aeruginosa*, *A. hydrophila* and *C. fimi* (Table 2). The highest inhibitory activity using the intracellular extract was observed against *P. aeruginosa*, followed by *S. aureus*, *A. hydrophila* and *C. fimi*. When the culture supernatant was tested, it was observed that the highest antimicrobial activity was against the indicator microorganism *L. monocytogenes* (8.7 mm). The smaller inhibition halos observed with both culture supernatant and intracellular extract were against *E. coli* (about 7.2 mm). Moreover, it was noted a positive antimicrobial activity in both culture supernatant and intracellular extract, against the indicator microorganisms *L. monocytogenes*, *E. coli*, *P. aeruginosa*, *A. hydrophila* and *C. fimi*.

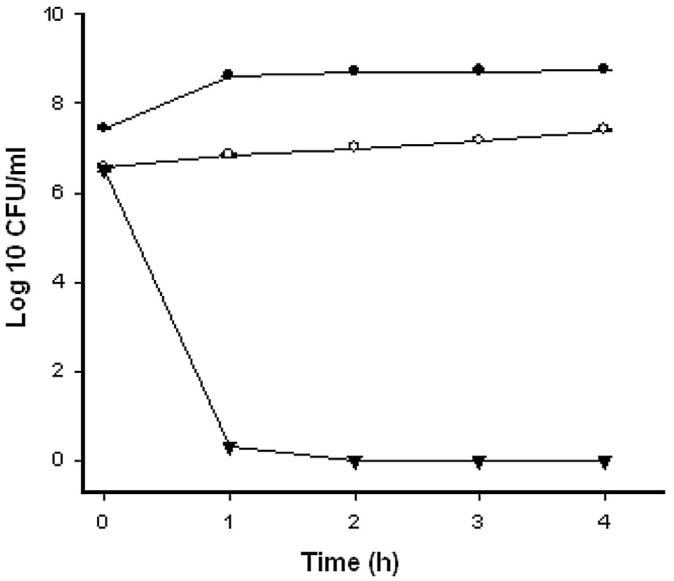

Fig. 2. Resistance of *E. durans* LAB18s to simulated gastric juice containing pepsin and acidified at pH 2.0 (▼) and 3.0 (○) after 4 h of incubation at 37 °C without shaking. The pH 7.0 without pepsin was used as control (●). Each point represents the mean ± s.e.m. of three independent experiments.

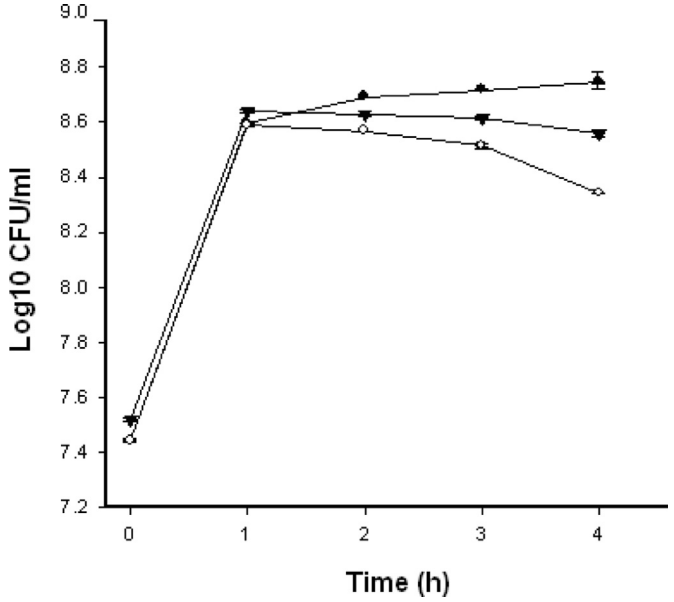

Fig. 3. Resistance to simulated intestinal juice of *E. durans* LAB18s containing pancreatin (pH 8) (●); pancreatin containing 1.5% bile salts (sodium cholate and sodium deoxycholate, 1:1) (▼); and medium at pH 7.0 without treatment as the control (○) after 4 h of incubation at 37 °C without shaking. Each point represents the mean ± s.e.m. of three independent experiments.

3.4. Antioxidant activity

The antioxidant activity was evaluated by three different methods: $ABTS^{\bullet+}$, DPPH and TBARS (Table 3).

The culture supernatant of *E. durans* LAB18s exhibited high ability to scavenge the radical $ABTS^{\bullet+}$, whereas the sample of intracellular extract showed weak antioxidant activity, with an inhibitory percentage of 9.4%. For DPPH method, the analysis conducted with culture supernatant showed high antioxidant activity ($EC_{50} = 3.6$) compared to the control ($EC_{50} = 9.77$). The intracellular extract of *E. durans* LAB18s showed low antioxidant activity ($EC_{50} = 8.79$) compared to the control ($EC_{50} = 9.48$).

When the culture supernatant and intracellular extract were evaluated by the TBARS method, it was observed that both samples showed high antioxidant activity as compared to the control (Table 3). These results indicate that *E. durans* LAB18s can be considered a bacterium with high antioxidant potential and may be useful to reduce the oxidative damage in food and feed.

Table 2
Antimicrobial activity using the culture supernatant and intracellular extract of *E. durans* LAB18s against indicator microorganisms.

Indicator microorganism	Inhibition zone (mm)[a]	
	Culture supernatant	Intracellular extract
L. monocytogenes	8.7 ± 0.3	7.3 ± 0.1
E. coli	7.2 ± 0.2	7.2 ± 0.1
B. cereus	nd[b]	7.5 ± 0.3
S. aureus	nd	9.1 ± 0.1
S. Typhimurium	7.5 ± 0.4	nd
S. Enteritidis	nd	7.3 ± 0.1
P. aeruginosa	7.5 ± 0.3	10.5 ± 0.1
A. hydrophila	7.8 ± 0.1	8.7 ± 0.6
C. fimi	7.3 ± 0.2	8.5 ± 0.3

[a] Values represent the mean ± s.e.m. of three independent experiments.
[b] Not detected; inhibition zones with values≤7 mm were assumed as absence of antimicrobial activity.

Table 3
Determination of antioxidant activity of the culture supernatant and intracellular extract of *E. durans* LAB18s by ABTS$^{\bullet+}$, DPPH and TBARS methods.[a]

Method	Control	LAB18s
ABTS$^{\bullet+}$ (%)		
Culture supernatant	100	71.9 ± 3.52
Intracellular extract	100	9.4 ± 0.21
DPPH (EC_{50} μg/ml)		
Culture supernatant	9.8 ± 0.01	3.6 ± 0.01
Intracellular extract	9.5 ± 0.08	8.8 ± 0.12
TBARS (nmol MDA/ml)		
Culture supernatant	0.875 ± 0.02	0.468 ± 0.01
Intracellular extract	0.755 ± 0.02	0.485 ± 0.09

[a] Values represent the mean ± s.e.m. of three independent experiments.

3.5. Growth and selenium bioaccumulation

E. durans LAB18s grew similarly in BHI medium in presence or absence of 15 mg/l sodium selenite, reaching the stationary phase after 6 h, with an OD_{600nm} around 1.4 (data not shown). The amount of selenium accumulated in the biomass reached 2.6 mg/g.

4. Discussion

E. durans LAB18s isolated from "Minas Frescal" cheese showed some desirable characteristics for a probiotic strain. *Enterococcus* species most commonly isolated from cheese are *E. faecalis* and *Enterococcus faecium*, followed by *E. durans* (Giraffa, 2003; Martín-Platero, Valdivia, Maqueda, & Martínez-Bueno, 2009). A high prevalence of enterococci in processed foods may be attributed to their resistance to heat, extreme salinity and harsh conditions during ripening of fermented foods (Gomes et al., 2008; Jurkovic et al., 2006). In contrast to human nutrition, where lactobacilli are common probiotics, *Enterococcus* spp. and *Saccharomyces* are frequently used as probiotics for animal nutrition (Lauková et al., 2008; Strompfová & Lauková, 2007). The major factors determining the survival of these bacteria include particular characteristics of the strains (e.g., acid and bile tolerance, and resistance to gastric and intestinal juice), composition of food ingested, and competition of microbiota in the intestine (Succi et al., 2005).

In order to select isolates with probiotic characteristics, the resistance to pH and bile salts is an importance factor in survival and growth of bacteria in the gastrointestinal tract. Results from this study showed that *E. durans* LAB18s has acid and bile tolerance, surviving to exposure in pH 3.0 and 4.0, and similarly, in all concentrations of bile salts tested (from 0.1% to 1.5%). Reports concerning the *in vitro* probiotic characteristics of enterococci are relatively scarce, in comparison with the abundant information on lactobacilli (Bhardwaj et al., 2010). The probiotic strain of *E. faecium* Fargo 688® could survive in the porcine gastric juice at pH 2.0 only for 8 min (Gardiner et al., 1999). The strain *E. faecium* SF68 retained viability and increased in number between 30 and 60 min of exposure to bovine bile, exhibiting an intrinsic tolerance towards bovine bile (Sun, Wang, & Jiang, 2010). When exposed to simulated gastric juice for 20 and 60 min, *E. faecium* SF68 exhibited a survival rate (62 and 56%, respectively) that would allow it to pass through the stomach. Also, this strain retained 92% viability when exposed to simulated small intestinal juice for 120 min, indicating that *E. faecium* SF68 can be classified as tolerant to the gastrointestinal secretions. In this study, the *E. durans* LAB18s survived in all times tested (1, 2, 3 and 4 h) at pH 3 and pH 4. In general, the acid tolerance of LAB depends on the pH profile of H^+-ATPase and the composition of the cytoplasmatic membrane. This is largely influenced by the type of bacterium, the composition of growth medium and the incubation conditions (Madureira et al., 2005).

High acidity in the stomach and the high concentration of bile components in the proximal intestine of the host, influence the selection of potential probiotic strains (Hyronimus et al., 2000). However, small intestine tolerance is potentially more important than gastric survival. With the development of new delivery systems and the use of specific foods, some studies indicate that acid-sensitive strains can be buffered through the stomach. However, to promote a positive effect to the host, probiotics need to survive and colonize the small intestine, and the condition of this environment may be an essential criterion for future probiotics (Huang & Adams, 2004).

In this study, the *E. durans* LAB18s demonstrated high ability to survive in the presence of simulated gastric juice containing pepsin (pH 3.0) and simulated intestinal juice containing pancreatin (pH 8, with or without addition of bile salts). The viability of *E. durans* LAB18s was also satisfactory when exposed to pH 3.0 and 4.0, although it was observed a decrease in viable cell counts in pH 2 even in the first hour. The pH of the stomach is between 2.5 and 3.5, although it may be lower during prolonged fasting (pH 1.5), or higher after a meal (pH 4.5) (Huang & Adams, 2004). Thus, the fact that the strain survived for a short time at pH 2.0 should not interfere with the probiotic ability, because it is intended to apply the strain concurrently with the feed, and thus the pH of the stomach is likely to be greater than 2.0. Thus, *E. durans* LAB18s survived to upper to gastrointestinal conditions and this tolerance to simulated gastric juice and simulated intestinal juice is very important to this strain be considered as an alternative source for future probiotic development.

The determination of hemolytic activity is considered a safety aspect for the selection of probiotic strains (FAO/WHO, 2002), and this activity was also investigated in this study. Our results were in agreement with those reported by Foulquié Moreno et al. (2003) on *Enterococcus* species (*E. faecium*, *E. faecalis*, *E. casseliflavus*, *E. durans*, *E. gallinarum* and *E. hirae*) isolated from different origins, showing that none of them exhibited hemolytic activity.

The antimicrobial and antioxidant activity of LAB has been related in the literature (González et al., 2007; Lin & Yen, 1999; Ou et al., 2009). The strain *E. durans* LAB18s showed high broad spectrum of antimicrobial activity, inhibiting *L. monocytogenes*, *E. coli*, *S.* Typhimurium, *P. aeruginosa*, *A. hydrophila* and *C. fimi* using the culture supernatant, and all indicator microorganisms tested (except for *S.* Typhimurium) using the intracellular extract. The antibacterial activity of LAB may be due to the production of organic acids, production of hydrogen peroxide, or bacteriocins (González et al., 2007). In the same way, a considerable number of strains belonging to different *Enterococcus* species display many interesting biotechnological properties such as proteolytic, lipolytic, esterolytic and other enzymatic activities, citrate utilization, and bacteriocin production (Belgacem et al., 2010; Foulquié Moreno et al., 2006). These characteristics are relevant to their technological performance, and suitable strains may be selected for application in food fermentations or as probiotic.

In this study, the antioxidant effects of *E. durans* LAB18s were observed by different methods, including scavenging of ABTS$^{\bullet+}$ and DPPH radicals, and TBARS method. The intracellular extracts of some LAB have metal ion chelating ability, reactive oxygen species scavenging ability, and reduction activity (Lin & Yen, 1999). Indeed, intact cells of LAB were found to possess antioxidant activity *in vitro* (Meira, Hetges, Velho, Lopes, & Brandelli, 2012; Ou et al., 2009). Nevertheless, using the intact cells as the delivery vehicles passing through the gastrointestinal tract, the intracellular constituents released from the LAB in gastrointestinal tract can be also antioxidative. Consumption of LAB containing foods or supplements may be recommended as healthy. It is well established that a wide variety of oxygen-centered free radicals and other reactive oxygen

species are continuously produced in the human body and in food systems (Lobo et al., 2010). Besides the long history of consumption, which proves the safety of consuming of LAB, they have been reported to have health-promoting characteristics that make these microorganisms desirable for use in the production of various health and functional foods.

In summary, the results obtained in this study suggest that *E. durans* LAB18s is a resistant strain to pass through the gastrointestinal tract. The viability of this strain through the exposure rate and the combination of simulated gastric juice and bile salts, intestinal juice, bile and acid tolerance were also observed. The *E. durans* LAB18s exhibited some desirable probiotic properties *in vitro*, such as antimicrobial activity and antioxidant ability, which were evidenced in both culture supernatants and intracellular extracts. Further investigations may be warranted to elucidate its potential health benefit and its application as promising probiotic strain in the feed industry.

Acknowledgments

This work received financial support of CAPES and CNPq (Brazil).

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