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ORIGINAL ARTICLE

Probiotic properties of *Lactobacillus plantarum* CECT 7315 and CECT 7316 isolated from faeces of healthy children

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Keywords
FAO/WHO guidelines, immune response, intestinal microbiota, *Lactobacillus plantarum* CECT 7315/7316, probiotic, safety.

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Abstract

Aims: Not all lactic acid bacteria possess the ability to confer health benefits for the host. Thus, it becomes necessary to screen and characterize numerous strains to obtain ideal probiotics. Here, two *Lactobacillus plantarum* strains (CECT 7315 and CECT 7316) were isolated and characterized.
Methods and Results: *In vitro* and *in vivo* tests were carried out for demonstrating the abilities as probiotics of CECT 7315/CECT 7316 *Lact. plantarum* strains. Both strains showed high ability to survive at gastro-intestinal tract conditions and to adhere to intestinal epithelial cells, as well as great inhibitory activity against a wide range of enteropathogens and ability to induce the production of anti-inflammatory cytokine IL-10.
Conclusions: *Lactobacillus plantarum* CECT 7315/CECT 7316 because of their potential probiotic properties could be excellent candidates for being tested in clinical trials aimed to demonstrate beneficial effects on human health.
Significance and Impact of the Study: Probiotics are live micro-organisms that confer a health benefit for the host. However, not all the lactic acid bacteria possess the ability to confer health benefits for the host. In this study, two *Lact. plantarum* strains (CECT 7315 and CECT 7316) were isolated and characterized to demonstrate their excellent qualities as potential probiotic strains.

Introduction

Probiotics are live micro-organisms that, when administered in adequate amounts, confer a health benefit for the host (Guarner *et al.* 2005). Probiotics act through a variety of mechanisms including the competition with potential pathogens for nutrients or adhesion sites, degradation of toxins, the production of antimicrobial substances and stimulating both the innate and the adaptive immune systems (Silva *et al.* 1987; Lewis and Freedman 1998; Isolauri *et al.* 2001).

There are a number of different organisms that can be classified as probiotics. The most common probiotic strains belong to *Lactobacillus* and *Bifidobacterium* genera. However, it has been observed that there is significant species and strain-specific variability in functional probiotic properties of lactobacilli (Sookkhee *et al.* 2001; Strahinic *et al.* 2007). Therefore, putative beneficial effect is strain dependent. Thus, it is essential that the potential probiotic strains are well characterized prior to their use.

As not all lactic acid bacteria possess the ability to confer health benefits for the host, it becomes necessary to screen and characterize numerous strains to obtain ideal probiotics. In this work, two *Lactobacillus plantarum* strains (CECT 7315 and CECT 7316) were isolated and characterized to demonstrate their qualities as potential probiotic strains.

Materials and Methods

Bacterial strains

Lactic acid bacteria strains were isolated from faeces of a healthy infant by plating on MRS (Oxoid, England) agar

supplemented with 0·05% (w/v) HCl, 100 μg l^{-1} novobiocin, 5 μg ml^{-1} nystatin, 5 μg ml^{-1} cycloheximide, 1 mg l^{-1} ampicillin and 10 μg ml^{-1} vancomycin (all Sigma-Aldrich, Steinheim, Germany). The final pH of the supplemented medium was 6·4. Plates were incubated at 30°C for 24 h in microaerophilic conditions (5% CO_2).

Lactobacillus rhamnosus GG, *Lactobacillus reuteri* ATCC 55730 and *Lact. plantarum* 299v strains were isolated from commercial Gefilus® (Valio Ltd, Helsinki, Finland), Reuteri Drops® (BioGaia, Stockholm, Sweden) and Jarro-Dophilus (Jarrow Formulas, Los Angeles, CA, USA), respectively. Strains of potentially pathogenic bacteria (*Salmonella enterica enteriditis* CECT 4155, *Salmonella enterica thypimurium* CECT 4594, *Bacillus subtilis* CECT 35, *Clostridium botulinum* CECT 4611 and *Yersinia enterocolitica* 10460) were obtained from the Spanish Collection of Type Cultures (CETC). A wild-type strain of *Escherichia coli* EHEC H7:O157 was also used. Lactic acid bacteria strains were grown in MRS plates for 24 h in microaerophilic conditions (5% CO_2). Pathogenic bacteria were grown in TSA plates incubated at 37°C for 24 h.

Genetic bacterial identification

Genomic DNA was extracted using Wizard genomic DNA purification kit (Promega Biotech Iberica, Madrid, Spain). The 16S gene was amplified by PCR using universal primers. DNA was washed using QIAquick PCR Purification kit (Qiagen, Hilden, Germany), and sequencing reactions were performed per sample, using primers fD1 and rP1described in Weisburg *et al.* (1991) and BigDye Terminator v.3·1 Cycle Sequencing kit, on a Genetic Analyzer 3130 (Applied Biosystems, Carlsbad, CA). DNA Sequence Analysis v.5·2 (Applied Biosystems) software was used to collect data, which were analysed through Chromas (Technelysium Pty Ltd.,) and BioEdit (Ibis Biosciences, Shannon, Ireland, IL, USA) software. Sequences data bases (NCBI Reference Sequences and Ribosomal Database Project) were used for bacteria species identification (http://www.ncbi.nlm.nih.gov/RefSeq/ and http://rdp.cme.msu.edu). Further strain genotyping was carried out according to a previously described protocol with minor modification (Rodas *et al.* 2005). Briefly, total DNA digestion was performed separately by *Sfi*I and *Sma*I restriction enzymes (Roche Diagnostics S.L., Barcelona, Spain), and Pulse-field electrophoresis (PFGE) was carried out.

Survival to gastro-intestinal tract conditions

The ability to survive to oral conditions was assessed by inoculating 200 μl of MRS medium (Sigma-Aldrich) with 5×10^7 CFU of each bacterial strain in 96-well culture plates. MRS was supplemented with lysozyme (Sigma-Aldrich) at 100, 200 and 300 μg ml^{-1} and with hydrogen peroxide (Sigma-Aldrich) at 10, 20 and 30 μg ml^{-1}. The lysozyme concentrations used were obtained multiplying by four, eight and 12 the initial amount described in Iacono *et al.* (1980), to study the survival of the strains in harder conditions. The hydrogen peroxide concentration was calculated taking in consideration that the strains could be in contact with a toothpaste containing 1–3% of hydrogen peroxide (SCCP, 2007). Plates were incubated at 37°C and 5% CO_2 for 1 h and 20 min for lysozyme and hydrogen peroxide, respectively. Bacterial growth was quantified by measuring optical density at 620 nm. Bacterial growth value was obtained by comparison with the growth achieved in standard MRS medium. The tolerance to acidic environments was assessed by adjusting MRS medium with HCl to pH 2, 3 and 4, while bile salt resistance was tested in MRS medium supplemented with 0·5 or 1% of Oxgall (Sigma-Aldrich, Spain) at pH 3, and with 0·3% of Oxgall in a nonacidic culture medium. Plates from acidic and bile salts assays were kept at 42 and 37°C (at 5% CO_2), and OD at 620 nm was measured 2 and 3 h after, respectively.

Adherence to intestinal epithelial cells

In vitro adhesion assay to intestinal pig mucosa was carried out as described by Collado *et al.* (2007). Briefly, pig mucosa was washed with PBS/0·01% gelatine/protease inhibitors cocktail (Complete®; Sigma-Aldrich) and dissolved in 10 mmol l^{-1} HEPES/HBSS/Complete® (P = 7·4). Each *Lact. plantarum* strains were incubated overnight with 5-3H-thymidine (1·0 μCi ml^{-1}; Amersham Biosciences, Buckinghamshire, UK)-labelled (10 μl ml^{-1}). The adhesion measurement was performed by the addition of 0·5 ml of radio-labelled-10^8 CFU of each strain to 24-h-preincubated mucus solution (0·5 mg ml^{-1} of protein) in a 24-well ELISA plate. After 60 min, supernatants of each well were carefully removed, three washes with PBS were performed and the mucus together with the adhering bacteria was scrapped. Recovered bacteria were lysed using 1% SDS in 0·1 mol l^{-1} NaOH by incubation at 60°C for 1 h to calculate specific radioactivity (cpm/CFU) and compared with commercial *Lact. rhamnosus* GG and *Lact. reuteri* strains. All assays were performed in triplicate.

Ability to antagonize pathogens

Salmonella enterica Enteritidis CECT 4155, *Salm. enterica Typhimurium* CECT 4594, *B. subtilis* CECT 35, *Cl. botulinum* CECT 4611, *Y. enterocolitica* NCTC 10460 and a wild-type strain of *E. coli* EHEC H7:O157 were used to evaluate antagonistic properties of *Lact. plantarum* CECT

7315/7316 strains. These pathogenic species were chosen because they are commonly present in the human gastrointestinal tract. The method used was an agar diffusion test with some modifications. Basically, *Lact. plantarum* CECT 7315 and CECT 7316 were grown on MRS plates at 37°C in microaerobic conditions (5% CO_2) to confluence. Six-millimetre-diameter cylinder sections of the confluents of *Lact. plantarum* CECT 7315 and CECT 7316 were obtained from the MRS plates. These cylinders were placed upside-down on McConkey plates inoculated with the pathogens and incubated overnight at 37°C in microaerophilic conditions (5% CO_2). Two independent experiments were performed, and the variables were tested in triplicate. The growth inhibitory activity (GI) was calculated subtracting the cylinder diameter (CD, cm) of the *Lact. plantarum* CECT 7315 and CECT 7316 cylinders from the inhibition zone diameter observed in the pathogen incubated plates (IZD, cm) as follows GI = (IZD − CD)/2.

Ability to induce the production of anti-inflammatory cytokines

THP-1 cells were obtained from the American Type Culture Collection (ATCC). Cells were grown in DMEM medium in 24-wells plates to a final concentration of 10^6 monocytes per well, approximately. The cells were stimulated by adding 10 ng ml^{-1} of lipopolysaccharide (LPS) during 150 min. Afterwards, cells were washed three times with PBS prior to their incubation with probiotic strains *Lact. plantarum* CECT 7315 and CECT 7316 in a proportion of 25 probiotic cfus per monocyte. The incubations were made in DMEM medium supplemented with gentamicin (50 μg ml^{-1}), ampicillin (10 μg ml^{-1}) and chloramphenicol (12 μg ml^{-1}) to avoid bacterial growth. Samples were taken every 6 h of incubation and at the end of the experiment (24 h). The commercial strains *Lact. reuteri* ATCC 55730 *and Lact. rhamnosus* GG were used as a positive control. Anti-inflammatory cytokine IL-10 levels were determined by flow cytometry using the BD™ Cytometric Bead Array System (BD Biosciences, Madrid, Spain) following manufacture's protocol. To interpret the results, the normalized slope between the values at 6 and 24 h was calculated by applying the following formula: 100 × [−(1–24 h IL-10)/6 h IL-10]/24. All the experiments were performed in duplicates.

Acute toxicity in rats

Eighteen 9-week-old Wistar rats were randomly distributed in three groups: *Lact. plantarum* CECT 7315, CECT 7316 and control. All animal procedures were in accordance with current European laws concerning animal experimentation. All protocols were approved by the Ethical Committee of the Universitat Autònoma de Barcelona and the Generalitat de Catalunya (protocols 605 and 3627, respectively). Animals were fed with Teklnad 2014 diet and water *ad libitum*. Two doses of 5×10^{10} CFU kg^{-1} of body weight dissolved in 1·5 ml of PBS in two consecutive days were administered to rats using oral probes, after eating over a full stomach. Control rats received 1·5 ml of PBS solution. Rats were weighed on days 0, 1 and every 2 days until they were killed. Total daily food and water consumptions were recorded. Rats well-being was determine each two days following a standardized morbidity protocol (data not shown). Animals with a score >7 were killed to avoid their suffering. Animals were killed at day 7 by CO_2 inhalation. Complete necropsies were carried out to detect possible organs and cavity macroscopic injuries. Furthermore, mesenteric lymph node samples were taken to determine whether bacterial translocation had occurred. Five milligrams of each sample was homogenized in 1 ml PBS containing 0·01% of gelatine, and 100 μl of these homogenates was seeded in McConkey plates (for assessing growth of enterobacteria) and MRS plates (for assessing growth of lactic acid bacteria). Plates were incubated at 37°C in microaerophilic conditions (5% CO_2) for 48 h. The positive cultures were defined as the appearance of any colony (even one) on the agar plates (Zhou *et al.* 2000). Experiments were performed in duplicates.

Antibiotic susceptibility

The minimum inhibitory concentration (MIC) was determined for the following substances: ampicillin, tetracycline, chloramphenicol, clindamycin, erythromycin and gentamicin. Probiotics strains were grown at 37°C in microaerophilic conditions (5% CO_2) for 24 h in LSM culture medium supplemented with the antimicrobials at different concentrations (ampicillin: 2 μg ml^{-1}, tetracycline: 32 μg ml^{-1}, chloramphenicol: 8 μg ml^{-1}, clindamycin: 1 μg ml^{-1}, erythromycin: 1 μg ml^{-1}, gentamicin: 18 μg ml^{-1}), except for gentamicin when the cells were incubated in TSB medium to avoid interferences of the medium in the measurements. The growth was calculated by measuring OD at 620 nm and comparing with the growth in the same medium without supplements. The strains were categorized as susceptible or resistant to antimicrobials according to the breakpoints levels described in a recent EFSA document (Bories *et al.* 2008).

Results

Genetic characterization

Sequences of a 1465-bp fragment of the 16S gene from CECT 7315 and CECT 7316 strains showed the highest

identity (98 and 99%, respectively) with *Lact. plantarum* WCFS1 when comparing with RefSeq and Ribosomal Database Project sequence databases. On the other hand, PFGE analyses showed that both strains are closely related (Fig. 1).

Survival to gastro-intestinal conditions and adherence to intestinal epithelial cells

Both *Lact. plantarum* strains had a growth in oral conditions between 80 and 140% and between 88 and 110% of the growth observed in standard MRS medium for lysozyme and hydrogen peroxide, respectively (Table 1). The relative growth in acidic conditions was between 46 and 83% of the basal growth, while increasing concentrations of bile acids led to a relative growth ranging between 54 and 180% of the growth in the standard medium.

Adhesion capacity of strains CECT 7315 and CECT 7316 was compared with those of *Lact. rhamnosus* GG and *Lact. reuteri* ATCC 55730 commercial strains. Strains *Lact. plantarum* CECT 7315 and CECT 7316 showed high adherence values (1·53 and 1·63 $\times$ 10^6 CFU cm^{-2}, respectively), doubling the positive controls (0·66 $\times$ 10^6 CFU cm^{-2} for *Lact. reuteri* and 0·71 $\times$ 10^6 CFU cm^{-2} for *Lact. rhamnosus* GG).

Ability to antagonize pathogens

Capacity of strains CECT 7315 and CECT 7316 to antagonize the growth of potentially pathogenic bacteria was

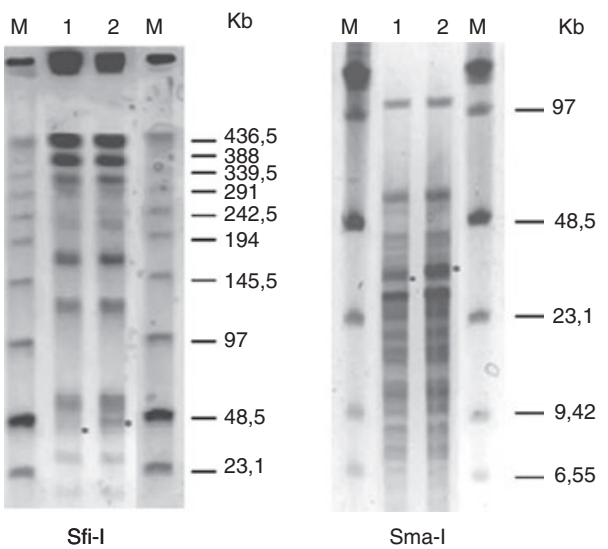

Figure 1 Genotype of *Lactobacillus plantarum* CECT 7315 (1) and CECT 7316 (2) strains. Genome digestion with *Sfi*I or *Sma*I of strain *Lact. plantarum* CECT 7316 yields an additional band when compared to strain CECT 7315 (stand out with an asterisk). DNA MW markers (M) were Lambda ladder PFG Marker and Low Range PFG Marker (New England Biolabs, Beverly, MA, USA) for *Sfi*I and *Sma*I digestions, respectively.

Table 1 Effect of lysozyme, hydrogen peroxide, pH and bile salts on growth of *Lactobacillus plantarum* CECT 7315 and CECT 7316

Condition	CECT 7315*	CECT 7316*
Lysozyme 100 μg ml^{-1}	140 ± 9%	80 ± 8%
Lysozyme 200 μg ml^{-1}	133 ± 16%	86 ± 9%
Lysozyme 300 μg ml^{-1}	136 ± 18%	95 ± 5%
Hydrogen peroxide 10 μg ml^{-1}	106 ± 10%	88 ± 6%
Hydrogen peroxide 20 μg ml^{-1}	110 ± 9%	107 ± 11%
Hydrogen peroxide 30 μg ml^{-1}	103 ± 10%	87 ± 7%
pH 2	46 ± 5%	54 ± 9%
pH 3	68 ± 6%	80 ± 8%
pH 4	65 ± 5%	83 ± 6%
0·3% bile salts, pH 6·5	54 ± 5%	126 ± 7%
0·5% bile salts, pH 3	77 ± 6%	172 ± 11%
1·0% bile salts, pH 3	73 ± 7%	180 ± 12%

*Results are expressed *vs* the control (%), which is the maximum growth of each of strains in a MRS standard broth.

Table 2 Inhibitory activity of *Lactobacillus plantarum* CECT 7315 and CECT 7316 strains, and *Lactobacillus rhamnosus* GG (LGG) against six pathogen strains

Pathogen strain	CECT 7315*	CECT 7316*	LGG*
E.coli EHEC, wild-type isolate	0·75	0·75	1·0
Salmonella enterica Enteriditis CECT 4155	1·0	1·0	1·0
Salm. enterica Typhimurium CECT 4594	0·5	1·0	0·75
Bacillus subtilis CECT 35	1·5	0·5	1·0
Clostridium botulinum CECT 4611	1·0	0·5	0·75
Yersinia enterocolitica 10460	2·0	2·5	1·0

*Values show the inhibitory activity (GI) calculated subtracting the cylinder diameter (CD, cm) from the inhibition zone diameter (IZD, cm) as follows: GI = (IZD − CD)/2

comparable to the wide-used *Lact. rhamnosus* GG probiotic strain, except for *Y. enterocolitica*, in which *Lact. plantarum* strains activity was higher (Table 2).

Ability to induce the production of anti-inflammatory cytokines

Lactobacillus plantarum CECT 7315 and CECT 7316 showed higher capacity to induce the production of anti-inflammatory cytokine IL-10 than the commercial strain *Lact. plantarum* 299v (Table 3). The immunomodulatory effect of the two strains of *Lact. plantarum* (CECT 7315 and CECT 7316) together was superior to the effect obtained by each strain alone, or when they were combined with *Lact. plantarum* 299v (Table 3).

Table 3 Ability of *Lactobacillus plantarum* CECT 7315 and CECT 7316 and 299v (L299v) strains to induce the production of the anti-inflammatory cytokine IL-10

Conditions	24 h IL-10 (pg ml^{-1})	6 h IL-10 (pg ml^{-1})	Slope	Normalized slope*
THP-1	0·945	0·805	0·01	0·72
THP-1 + ANT	1·05	0·915	0·01	0·59
THP-1 + ANT + LPS	33·1	18·0	0·63	3·48
THP-1 + ANT + LPS + CECT 7315	234·5	27·8	8·61	31·04
THP-1 + ANT + LPS + CECT 7316	283·7	31·4	10·51	33·53
THP-1 + ANT + LPS + CECT 7315 + CECT 7316	348·3	28·1	13·34	47·46
THP-1 + ANT + LPS + CECT 7315 + L299v	423·9	45·5	15·77	34·68
THP-1 + ANT + LPS + CECT 7316 + L299v	270·8	32·3	9·94	30·82
THP-1 + ANT + LPS + L299v	232·9	37·9	8·13	21·42

THP-1: monocyte cell line obtained from the American Type Culture Collection (ATCC); ANT: antibiotics (gentamicin (50 μg ml^{-1}), ampicillin (10 μg ml^{-1}) and chloramphenicol (12 μg ml^{-1}); LPS: lipopolysaccharide (10 ng ml^{-1}).
*Normalized slope was calculated as follows: 100 × [–(1–24 h IL-10)/6 h IL-10]/24.

Acute toxicity in rats

Neither clinical symptoms nor alteration of the animals' well-being were detected along the study. All the animals showed similar body weight evolution along the study, and no significant difference among groups in diet and water consumptions was recorded (data not shown). Animal' necropsies and histopathological examinations showed no macroscopic damages in organs and cavities. No enterobacteria translocation occurred in any group. In contrast, some lactic acid bacteria were isolated from mesenteric lymph node from animals of the three groups (Table 4).

Antibiotic susceptibility

Lactobacillus plantarum CECT 7315 and 7316 were susceptible to all the antimicrobials tested (data not shown).

Table 4 Bacterial translocation to mesenteric lymph node from rats inoculated with *Lactobacillus plantarum* CECT 7315 and CECT 7316 or a PBS solution (control group)

Group	Sex	Enterobacteria*	Lactic acid bacteria*	Maximum translocation observed (CFU/mg)
Control	Males	0/3	2/3	2
	Females	0/3	2/3	2
CECT 7315	Males	0/3	1/3	2
	Females	0/3	2/3	4
CECT 7316	Males	0/3	1/3	4
	Females	0/3	0/3	0

*Number of animals with positive bacterial growth (appearance of any colony)

Discussion

The isolation and characterization of novel probiotic strains more effectively and with better probiotic characteristics than those already commercialized strains is important to satisfy the increasing request of the market. To claim that a bacterial strain is a potential probiotic strain, the use and adoption of several guidelines suggested by a joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) working group should be a prerequisite. In this work, these guidelines were followed for demonstrating the abilities as probiotics of two *Lact. plantarum* strains (CECT 7315 and CECT 7316). These strains were isolated from 0- to 5-year-old children mostly fed with vegetables. The human origin of the probiotic strains could increase the possibility of success because a probiotic strain can function better in a similar environment to where it was originally isolated from (Saarela *et al.* 2000).

By sequencing nearly complete 16S gene, the strains were assigned to *Lact. plantarum* species. The strains were also typed by analysing their PFGE profiles, the gold standard method proposed by the FAO/WHO guidelines.

Subsequently, the strains were subjected to characterization for their functionality. The excellent capacity that both *Lact. plantarum* CECT 7315 and CECT 7316 strains possess to survive in acidic pH and in lysozyme and bile concentrations similar to that present in the oral and gut conditions and their adhesion to intestinal mucus was confirmed. Both abilities have been suggested as important prerequisites for probiotic action (Saarela *et al.* 2000; FAO/WHO, 2002). The capacity to overcome gastrointestinal conditions ensures that a high proportion of probiotic cells arrive alive to the intestine, where they

exert their function. *Lactobacillus plantarum* CECT 7315 seems to be better than CECT 7316 (Table 1) in surviving the gastrointestinal conditions for lysozyme and hydrogen peroxide. However, *Lact. plantarum* CECT 7316 survived better in acidic condition and supplemented with bile salts. These results seem to indicate different and independent resistance mechanisms for these adverse conditions. On the other hand, adhesion to intestinal epithelial cells provides an interaction with mucosal surface facilitating the contact with gut-associated lymphoid tissue mediating local and systemic immune effects (Salminen *et al.* 1996) and provides means of competitive exclusion of pathogenic bacteria from the intestinal epithelium (Bernet *et al.* 1993, 1994; Coconnier *et al.* 1993a,b).

Moreover, *Lact. plantarum* CECT 7315 and CECT 7316 showed high capacity, even higher than *Lact. rhamnosus* GG commercial strain, to inhibit a wide range of enteric pathogens, including gram-negatives such as *E. coli*, *Salm. enterica* and *Y. enterocolitica* and gram-positive pathogens such as *B. subtilis* and *Cl. botulinum*. This antagonism against pathogenic bacteria, because of the production of antimicrobial substance or by competitive exclusion, is important to have an impact on the colonic flora by promoting the establishment of a beneficial microbiota. Owing to this broad inhibitory spectrum activity, the antagonism activity might be attributed to the production of low molecular weight metabolites (such as hydrogen peroxide, lactic and acetic acid) rather than to the production of bacteriocins, which usually show an inhibitory effect only against closely related species (Holzapfel *et al.* 1995). However, further analyses should be performed to determine the mechanism by which *Lact. plantarum* CECT 7315 and CECT 7316 exert their inhibitory effect.

It was also demonstrated that both probiotic strains induce the production of anti-inflammatory cytokines (IL-10), especially when they are administered together. Interleukin-10 has pleiotropic effects in immunomodulation and inflammation, being an essential immunoregulator in the intestinal tract, able to inhibit the synthesis of pro-inflammatory cytokines as well as to suppress the antigen presentation capacity of antigen presenting cells (Grimbaldeston *et al.* 2007). On the other hand, IL-10 enhances B cell survival, proliferation and antibody production. However, the possible benefits of the consumption of *Lact. plantarum* CECT 7315 and CECT 7316 on human immune system must be elucidated in well-designed clinical trials.

Finally, although *Lact. plantarum* species has the QPS status (Qualified Presumption of Safety) by EFSA based on the history of apparent safe use, the safety of both strains was assessed by performing an acute toxicity assay in rats. It was demonstrated that both strains are safe even when they are administered at high doses, two orders of magnitude higher than the dose expected to be administered to humans. Although some kind of lactic acid bacteria translocation was observed, this phenomenon could be attributed to normal basal translocation of lactic acid bacteria (Zhou *et al.* 2000).

Lactobacillus plantarum CECT 7315 and CECT 7316 followed the EFSA technical guidelines related to assessment of bacterial resistance to antibiotics (Bories *et al.* 2008).

In summary, this study demonstrated that *Lact. plantarum* CECT 7315 and CECT 7316 possess good functional probiotic properties like high tolerance to oral-gastrointestinal tract as well as antimicrobial activity against the most common enteropathogens and ability to induce the production of anti-inflammatory cytokines. However, the ability of these strains to improve human health must be determined in well-designed controlled clinical trials.

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