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Simulated gastrointestinal conditions increase adhesion ability of *Lactobacillus paracasei* strains isolated from kefir to Caco-2 cells and mucin

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ABSTRACT

Gastrointestinal conditions along the digestive tract are the main stress to which probiotics administrated orally are exposed because they must survive these adverse conditions and arrive alive to the intestine. Adhesion to epithelium has been considered one of the key criteria for the characterization of probiotics because it extends their residence time in the intestine and as a consequence, can influence the health of the host by modifying the local microbiota or modulating the immune response. Nevertheless, there are very few reports on the adhesion properties to epithelium and mucus of microorganisms after passing through the gastrointestinal tract. In the present work, we evaluate the adhesion ability *in vitro* of L. *paracasei* strains isolated from kefir grains after acid and bile stress and we observed that they survive simulated gastrointestinal passage in different levels depending on the strain. *L. paracasei* CIDCA 8339, 83120 and 83123 were more resistant than *L. paracasei* CIDCA 83121 and 83124, with a higher susceptibility to simulated gastric conditions. Proteomic analysis of *L. paracasei* subjected to acid and bile stress revealed that most of the proteins that were positively regulated correspond to the glycolytic pathway enzymes, with an overall effect of stress on the activation of the energy source. Moreover, it is worth to remark that after gastrointestinal passage, *L. paracasei* strains have increased their ability to adhere to mucin and epithelial cells *in vitro* being this factor of relevance for maintenance of the strain in the gut environment to exert its probiotic action.

1. Introduction

Exposure to gastric and intestinal conditions along the digestive tract fluids is the main stress that might affect the viability of ingested probiotics microorganisms (Liong & Shah, 2005). If probiotic strain is intended for oral administration, it must survive the adverse conditions of the gastrointestinal tract (GIT) to reach the site of action alive: the small and large intestine. The tolerance for acidity, bile and pancreatin *in vitro* predict the behaviour of lactic acid bacteria in the conditions found in the GIT (WHO/FAO, 2006).

Adhesion to epithelium is a prerequisite for bacterial colonization and has been considered one of the main criteria for the characterization of probiotic bacteria (Tuomola, Ouwehand, & Salminen, 2000) since adhered bacteria extends their residence time in the intestine improving their influence on the host health either by modifying the local microbiota or modulating the immune response. Adhesion experiments *in vivo* are difficult to carry out, therefore several *in vitro* models have been developed to evaluate this property. These methods are based on the use of matrices such as mucus membrane proteins (collagen, laminin, fibronectin, etc.) and different model intestinal

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epithelium as Caco-2 cell line.

Epithelial cells of the gastrointestinal tract are covered by a layer of mucus that protects the epithelium from mechanical damage, pathogens, and also provides habitat and nutrients to the intestinal microflora (Tuomola, Ouwehand, & Salminen, 1999). Mucus is the first barrier in the intestine facing microorganisms; therefore, adhesion to this matrix is considered a requirement for colonization of the gut (Ouwehand, Kirjavainen, Grönlund, Isolauri, & Salminen, 1999). The main components of mucus are polymeric glycoproteins called mucins that have been widely used for testing *in vitro* adhesion of probiotics (Matsumoto, Tani, Ono, Ohishi, & Benno, 2002; Tuomola et al., 2000). The mechanisms by which adherence to mucus is achieved are poorly understood. However, a decrease in bacteria adhesion after treatment with proteases has been described, indicating that bacterial surface proteins are involved in the process (Lorca, Torino, de Valdez, & Ljungh, 2002; Tuomola et al., 2000). Furthermore, purified surface proteins are able to adhere to mucus or epithelial cells (Roos & Jonsson, 2002; Sillanpää et al., 2000). Many of them have glycosidic components in its structure. For example, the strain L. *kefiri* 8348 has a glycosylated S-layer (Mobili et al., 2009) and has the capacity to adhere to mucus (Carasi et al., 2014) and epithelial cells (Golowczyc, Mobili, Garrote, Abraham, & De Antoni, 2007). Moreover, *L. plantarum* adhesion to HT-29 cells is a strain-dependent property and is mediated by mannose (Adlerberth et al., 1996).

L. paracasei strains studied in this work are polysaccharide producing bacteria (Hamet et al., 2013), in consequence is feasible to think the intervention of carbohydrate components in adhesion properties. Most published studies evaluate the survival to GIT simulated conditions and adherence to epithelial cells independently. However, there are very few results on the properties of adhesion to epithelium and mucus of microorganisms after passing through the GIT. Since the evaluation of lactobacilli adhesiveness after subjecting to the gastrointestinal stress can provide a suitable model that approaches to physiological conditions, the aim of the present work was to evaluate adhesion ability of L. *paracasei* strains isolated from kefir grain after acid and bile stress.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Lactobacillus paracasei CIDCA 8339, 83120, 83121, 83123 and 83124, isolated from kefir grains (Hamet et al., 2013) were cultured in MRS-broth (DIFCO, Detroit, USA) at 30 °C for 48 h in aerobic conditions. Frozen stock cultures were stored at − 80 °C in skim milk.

2.2. Resistance of lactobacilli to simulated gastrointestinal conditions

The simulated gastric and intestinal fluids solutions were prepared as described by Grimoud et al. (2010). Simulated gastric juice (NaCl 125 mM, KCl 7 mM, $NaHCO_3$ 45 mM, pepsin 3 g/L) at a final pH adjusted to 2.5 was inoculated with a 48 h bacteria culture at a final concentration of 10^8 CFU/mL. Suspensions were incubated at 37 °C with stirring at 200 rpm. After 90 min of incubation, bacteria were washed with PBS in phosphate-buffered saline (PBS) pH 7.0 and the pellets were suspended in simulated intestinal fluid (pancreatin 0.1% *w/v*, bovine bile salts 0.15% *w/v*) at a final pH adjusted to 8.0 for 3 h in the same conditions. Bacterial viability was assessed by plating samples collected after incubation in simulated gastric fluid and after incubation in simulated intestinal fluid in MRS agar.

2.3. Adhesion assays

2.3.1. Bacterial binding to mucin

Plate preparation. Partially purified type III porcine gastric mucin obtained from Sigma-Aldrich (hereafter referred to as MUCIN) was dissolved in PBS. Solutions of 3 g/L MUCIN were bound to 96-well sterile polystyrene plates (Maxisorp Nunc, Roskilde, Denmark), according to Carasi et al. (2014). Briefly, plates were incubated at 37 °C for 1 h, followed by an overnight incubation at 4 °C. A second incubation for 2 h at 37 °C was performed with the same solution in order to minimize the number of empty binding sites in the polystyrene microtitre plates. Finally, the wells were washed twice with 200 µL of PBS.

Binding assay. Bacterial preparation was performed according to Tallon, Arias, Bressollier, and Urdaci (2007). *L. paracasei* strains were grown for 48 h at 30 °C in MRS-broth (DIFCO, Detroit, USA). Aliquots of 1 mL were sampled and centrifuged at 10000 × *g* at 10 °C for 5 min and the pellets were washed twice with sterile PBS, suspended in the same buffer and adjusted to the optical density OD^{550} = 1.0 (around 10^9 CFU/mL). One hundred microliters of the bacterial suspension were added to each well. The plates were incubated 2 h at 37 °C. The wells were washed 6 times with 200 µL of sterile PBS to remove unbound bacteria and then treated with 200 µL of a 5 mL/L Triton X-100 solution for 30 min at 37 °C to desorb the bound bacteria. 100 µL of the content of each well were removed, diluted in PBS, plated on MRS agar plates. Colony counts were performed after 48 h at 30 °C. The concentration of Triton X-100 and the temperature of contact used were tested on all strains in order to determine its influence on bacterial viability. A minimum of four replicates were used to estimate the adhesion of a given strain. Each replicate corresponds to an independent culture that was tested in duplicate each time.

2.3.2. Bacterial binding to Caco-2/TC-7 cell line

Caco-2/TC-7 cells were routinely grown following the procedure described by Minnaard et al. (2007). For adhesion assay, Caco-2/TC-7 monolayers were incubated with 0.25 mL of *Lactobacillus* suspension (2×10^8 CFU/mL) and 0.25 mL DMEM (GIBCO BRL Life Technologies Rockville, USA) for 1 h at 37 °C in a 5% CO_2 – 95% air atmosphere. Then, the monolayer was washed three times with PBS and lysed by adding sterile distilled water. To determine the number of viable bacterial cells associated to Caco-2/TC-7 cells, appropriate dilution performed in 0.1% p/v tryptone were plated on MRS agar and colony counts were performed. A minimum of four replicates were used to estimate the adhesion of a given strain. Each replicate corresponds to an independent culture that was tested in duplicate each time.

2.4. Two-dimensional electrophoresis (2-DE)

Treated and control L. *paracasei* CIDCA 83123 were suspended in 400 µL of 30 mM Tris-HCl pH 8.0 containing 7 M urea, 2 M tiourea, 4% *w/v* CHAPS and 2% w/v ASB14. Bacterial suspensions were sonicated for 1 min with pulses every 0.5 s in a Digital Sonifier® S250D (Branson, USA) and then were centrifuged at 16000 *g* for 5 min. Supernatants were treated with a 2D Clean-up kit (GE Healthcare, USA), and precipitated proteins from each sample were resuspended in 100 µL of 30 mM Tris-HCl pH 8,5 with 8 M urea, 2,5% *w/v* CHAPS and 2% w/v ASB14, and then quantified by BCA Protein Assay (Pierce™, ThermoFisher Scientific, USA). Each sample (30 µg of total protein) was seeded into the strips, and first dimension (isoelectrofocusing) was runned in Ettan™ IPGphor 3 equipment, using a 3–10 ampholites range (time: 8 h, total voltage: 16,525 V). After first running, strips were treated with 10 mg/mL DTT to reduce disulfide groups, and then treated with 25 mg/mL iodoacetamide in order to alkylate free sulfhydryls. The second dimension (SDS-PAGE) was runned in 12% gels using a BioRad Mini-Protean Tetra (BioRad Laboratories, Richmond, CA, USA) equipment according to Laemmli (1970). Bench Mark Protein Ladder (GE Healthcare) was used as molecular weight reference. The gels were revealed using Colloidal Blue staining (Neuhoff, Arold, Taube, & Ehrhardt, 1988) and selected spots were excised using a fresh scalpel and diced into 1 mm^3 pieces.

Gels were destained with distilled water and digitized with an Umax Astra 4000 U scanner (UMAX Technologies, Dallas TX 75243, U.S.A.) at

Table 1
Proteins from *L. paracasei* CIDCA 83123 that were differentially abundant in response to acid and bile stress and putative function.

Spot number	MW[a] (KDa)	pI[b]	Fold intensity ratio (Lp123-GIstress[c]/ Lp123)	Accession number[d]	Homologous protein and/or function (Organism)
1	41	4.34	0.08	gi|511750863 (WP_016385551.1)	Cell wall hydrolase, amydase family (*L. paracasei*)
2	39	5.99	0.22	gi|116493594 (YP_805328.1)	Surface antigen (*L. casei* ATCC 334)
				gi|511699420 (WP_016379272.1)	Surface antigen (*L. paracasei*)
3	36	7.15	0.59	gi|116494473 (YP_806207.1)	Glyceraldehyde-3-phosphate dehydrogenase (*L. casei*, *L. paracasei*)
				gi|511645614 (WP_016364194.1)	
4	29	8.09	0.53	gi|511665660 (WP_016372423.1)	Protein lacX, plasmid, partial (*L. paracasei*)
				gi|511741377 (WP_016380947.1)	Galactose mutarotase-like protein, partial (*L. paracasei*)
5	75	8.51	0.26	gi|518733420 (WP_019893263.1)	Cell wall-associated hydrolase, partial (*L. paracasei*)
6	36	7.25	1.69	gi|116494473 (YP_806207.1)	Glyceraldehyde-3-phosphate dehydrogenase (*L. casei*, *L. paracasei*)
				gi|511645614 (WP_016364194.1)	
7	36	7.48	2.7	gi|116494473 (YP_806207.1)	Glyceraldehyde-3-phosphate dehydrogenase (*L. casei*, *L. paracasei*)
				gi|511645614 (WP_016364194.1)	
8	35	7.83	4.3	gi|511658911 (WP_016367520.1)	L-lactate dehydrogenase partial (*L. casei*, *L. paracasei*)
				gi|489692047 (WP_003596230.1)	
10	25	7.45	1.4	gi|511648232 (WP_016365983.1)	Phosphoglyceromutase (*L. paracasei*, *L. casei*)
				gi|116495592 YP_807326.1)	
11	34	6.14	2.6	gi|191638032	UTP-glucose-1-phosphate uridylyltransferase (*L. casei* BL23)

[a] Molecular mass (KDa).
[b] Calculated isoelectric point.
[c] GIstress: gastrointestinal stress.
[d] Sequence identification number in the NCBInr database.

resolution of 300 dpi. Image analysis and statistical quantification of relative protein levels were performed using Progenesis SameSpots V.4 (Nonlinear Dynamics, Newcastle, UK) by pairwise comparison (Table 1 shows the detailed statistics of each spot). A protein was considered differentially expressed if the mean normalized spot volume varied at least 1.4-fold between compared spots. At least three biological replicates for each growth condition were performed.

2.5. Mass spectrometry (MALDI-TOF)

In-gel protein digestion. Gel pieces were destained in 3 × 100 µL washes of 25 mM NH_4HCO_3, 5% acetonitrile (ACN) (pH 8,5), followed by reduction with 100 µL 10 mM DTT in 25 mM NH_4HCO_3 at room temperature for 1 h and then alkylation with 25 mM iodoacetamide in 25 mM NH_4HCO_3, at 37 °C for 1 h. Gel pieces were desiccated with 100 µl of ACN 100% for 10 min at room temperature and rehydrated with 30 ng of trypsin (Promega Trypsin Gold, TPCK treated) in 25 mM NH_4HCO_3. The enzymatic reaction was carried out at 37 °C for 3 h and peptides were extracted from the gel pieces with 100 µL of 50% ACN, 0.2% TFA. The eluted peptides were dried with a Speed-Vac™ and then suspended in 5 µL of 0.1% TFA. All assays were carried out in MultiScreen solvinert filter plate (Millipore) with a MultiScreen™ Vacuum Manifold 96-well system (MILLIPORE, Billerica, MA).

MALDI-TOF-MS analysis. Peptide mass fingerprint (PMF) analysis was used to determine protein identities. PMF spectra were acquired with an ultrafleXtreme MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) at the SePBioEs Proteomics Service (UAB, Barcelona, Spain). Samples were spotted on a ground steel target plate (Bruker Daltonics) mixing 0.5 µL of each sample with 0.5 µL of freshly prepared matrix solution of 10 mg/mL α-cyano-4-hydroxycinnamic acid (Bruker Daltonics) in a 30% ACN and 0.1% TFA aqueous solution. An external calibration was performed using a standard peptide mixture (Bruker Daltonics). Peptide masses were acquired within a range of *ca. m/z* 800–4000. Protein identification was carried out with Mascot search engine (Matrix Science Inc., Boston, MA) using the following parameters: two missed cleavages, 100 ppm tolerance, cysteine carbamidomethylation and methionine oxidation were set as variable modifications. Searches were performed using the NCBInr database restricted to Firmicutes.

2.6. Statistical analysis

One way analysis of variance (ANOVA) was performed. Differences were statistically tested using Tukey's multiple comparison test ($p < 0.05$) conducted by the GraphPad Prism® software.

3. Results

3.1. Survival of Lactobacillus paracasei strains after simulated gastrointestinal passage

The ability of five *L. paracasei* strains to survive to the simulated gastrointestinal conditions was assessed. Fig. 1 shows the obtained results. In all cases, the critical step was the simulated gastric passage which lowered viability significantly ($p < 0.05$), at least 1.5 logarithmic units. After complete simulation, the viability resulted ranged from 1.02×10^3 to 2.46×10^6 CFU/mL (with an initial inoculum of 1×10^8 CFU/mL), depending on the strain. Among the studied strains, *L. paracasei* CIDCA 8339 was the most resistant to simulated gastric and intestinal fluids whereas *L. paracasei* CIDCA 83121 and CIDCA 83124 were very sensitive to this treatment, showing a viability decrease of almost 5 logs.

3.2. Adhesion to Caco-2 cells and mucin after simulated gastrointestinal passage

Taking into account that all these strains were able to adhere to the

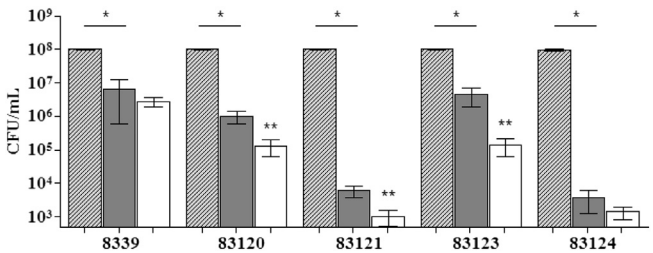

Fig. 1. Survival of *L. paracasei* strains after passage through simulated gastric (▭) and subsequent intestinal (▭) conditions. Initial bacterial counts are shown in dashed bars (▨). CFU/mL: colony forming units per mililiter. *P < 0.05 vs initial bacterial count; **P < 0.05 vs gastric conditions.

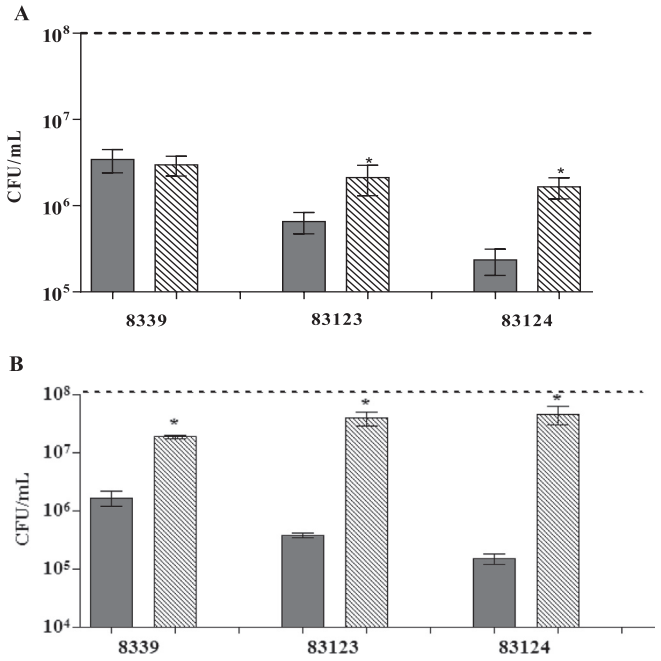

Fig. 2. Adhesion to Caco-2 cells (A) and porcine gastric mucin (B) of *L. paracasei* strains before (▭) and after (▨) passage through gastrointestinal conditions. CFU/mL: colony forming units per militer. *P < 0.05.

intestinal epithelial cell line Caco-2/TC-7 (6–7 log CFU/cell when 100 lactobacilli per cell or 1.0×10^8 CFU/mL were initially inoculated) (Zavala et al., 2016), three *L. paracasei* strains were selected to evaluate the effect of gastric and intestinal stress on adhesion properties to Caco-2/TC-7 cells and porcine gastric mucin (Fig. 2).

Regarding adhesion to Caco-2/TC-7 cells, gastrointestinal passage significantly ($p < 0.05$) increased the adhesion of *L. paracasei* CIDCA 83123 and 83124 in 0.5 and 1 log cycles, respectively. However, adhesion capacity of *L. paracasei* CIDCA 8339 was not affected (Fig. 2a).

The three strains had the ability to adhere to porcine gastric mucin in an order of 1.50×10^5 to 1.67×10^6 CFU/mL, when 1.0×10^8 CFU/mL were initially inoculated without treatment. It is noteworthy that gastrointestinal tract's passage simulation significantly increased ($p < 0.05$) adhesion to mucin of *L. paracasei* CIDCA 8339, 83123 and 83124 indicating that gastric and intestinal stress causes changes in bacterial surface that led to an increased ability to adhere to the epithelium.

3.3. Proteomic analysis

3.3.1. Bidimensional electrophoresis

Based on both its resistance to gastrointestinal simulation and its adhesion properties, *L. paracasei* CIDCA 83123 was selected for proteomic analysis in order to study the changes induced by gastrointestinal passage. Whole cell protein extracts were obtained by lysis of bacteria before and after simulated gastrointestinal passage. Two-dimensional electrophoresis (2-DE) was performed after precipitation of the proteins. Gels showed some clear differences between the sample that was subjected to treatment (Lp123-GIstress) and the untreated bacteria (Lp123). When lactobacilli underwent the gastrointestinal conditions *in vitro*, 8 selected spots were more intense, while 5 decreased their intensity compared to untreated control (Fig. 3-Table 1). The thirteen spots corresponding to differentially expressed proteins were selected to be identified by MALDI/TOF MS - PMF.

3.3.2. Mass spectrometry identification (MALDI-TOF/MS - PMF)

Among the five proteins that were down regulated, three of them could be associated to surface proteins of *L. paracasei* and the other two proteins were related to sugar metabolism (glyceraldehyde-3 phosphate dehydrogenase and galactose mutarotase-like protein). Among the eight spots with greater intensity after GI treatment, three of them (spots 9, 12 and 13 of Fig. 3) could not be identified. The five up regulated proteins that were identified corresponded to isoforms of glycolytic pathway enzymes or enzymes involved in monosaccharide's activation (Table 1). Two of these spots corresponded to isoforms of the glyceraldehyde 3-phosphate dehydrogenase enzyme. Another one was identified as a fragment of L-lactate dehydrogenase which participates in obtaining pyruvate from lactate. Another spot corresponded to phosphoglyceromutase, an enzyme also involved in the glycolytic pathway. Finally, UTP glucose 1-phosphate uridyltransferase, that is involved in sugar activation was also identified.

4. Discussion

The intrinsic tolerance to gastrointestinal stress factors is a key element in guaranteeing the performance of probiotics since a large number of viable microorganisms must reach the intestine in order to produce a beneficial effect on the consumers' health (De Angelis & Gobbetti, 2004). Therefore, enteric bacteria, including lactobacilli and bifidobacteria, must have developed specific mechanisms to resist the harmful action caused by acidity in the stomach or bile in the gut. According to our results the five L. *paracasei* strains isolated from kefir survive simulated gastrointestinal passage in a strain dependent way being *L. paracasei* CIDCA 8339, 83120 and 83123 more resistant than L. *paracasei* CIDCA 83121 and 83124. The higher decrease in viability was observed after exposure to simulation of the gastric conditions indicating that acid treatment exerted more damage to these strains than bile treatment or the acid stress exerted a selection toward bile salt-resistance microorganisms (Chou & Weimer, 1999).

Lactic acid bacteria employ various mechanisms in response to acid stress, including the maintenance of intracellular pH (Wu, He, & Zhang, 2014), preservation of cell membrane functionality (Wu, Zhang, Wang, Du, & Chen, 2012) or induction of stress response proteins (Hamon, Horvatovich, Marchioni, Aoudé-Werner, & Ennahar, 2014). Results of proteomics analysis indicated that multiple metabolic pathways might have been involved in the adaptation to acid stress including carbohydrate metabolism, signal transduction mechanisms, general functions, translation system, chaperones, nucleotide transport and metabolism, and amino acid biosynthesis (De Angelis & Gobbetti, 2004). It has been proven that overexpression of proteins involved in carbohydrate metabolism plays an important role in some strains of *L. casei* by supplying energy to resist acid stress (Wu et al., 2011, 2014). In addition, Nezhad, Knight, and Britz (2012) demonstrated that some enzymes involved in glycolysis were up-regulated in the cell surface fraction of *L. casei* grown at low pH, including enolase, lactate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase.

Development of bile resistance involves a variety of processes addressed toward detoxification of bile and counteracting the deleterious effect of the environment on bacterial structures. Active efflux of bile acids/salts, bile salt hydrolysis, exopolysaccharides (EPS) and/or surface proteins production and changes in the architecture/composition of cell membrane appear to be the most prevalent bile-specific mechanisms mediating resistance in *Lactobacillus* and *Bifidobacterium* (Bustos, Raya, de Valdez, & Taranto, 2011; Ruiz, Margolles, & Sánchez, 2013).

Proteomic analysis of *L. paracasei* CIDCA 83123 subjected to both acid and bile stress revealed that most of the proteins that were positively regulated correspond to the glycolytic pathway enzymes, with an overall effect of stress on the activation of the energy source. The cytoplasmic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) takes part in the glycolysis; however many proteins of the carbohydrate metabolism are often associated with the cell wall where they are able to exert other functions. These proteins, called

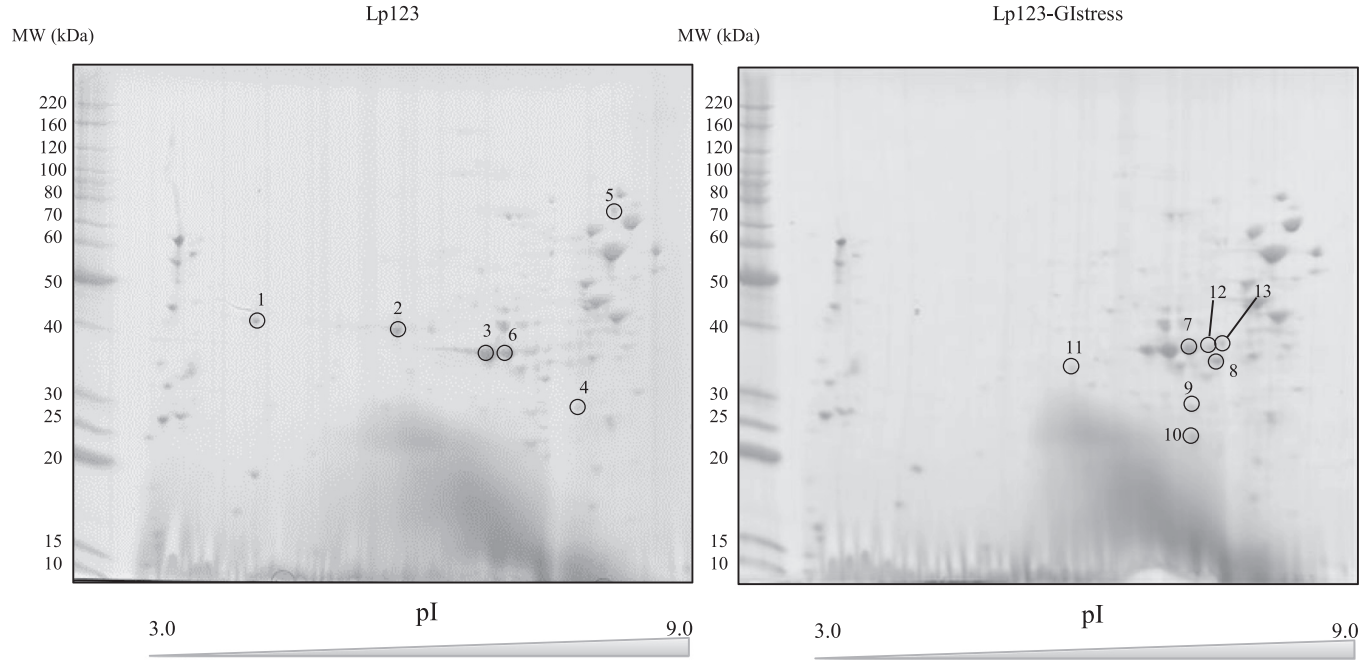

Fig. 3. Two-dimensional eletrophoresis (2DE) of total proteins from *L paracasei* CIDCA 83123 before (Lp123) and after (Lp123-GIstress) passage through simulated gastrointestinal conditions. MW: molecular weight; pI: isoelectric point. Numbered spots were analyzed by mass spectrometry (MALDI-TOF).

"Moonlighting proteins", could improve the contact of the bacteria with the epithelial cells or contribute to the degradation of the extracellular matrix, thus facilitating the colonization of the human gut (González-Rodríguez et al., 2012; Jeffery, 2017).

It is worth to remark that after gastrointestinal passage all the *L. paracasei* strains isolated from kefir, have increased their ability to adhere to mucin and epithelial cells *in vitro* being this factor of relevance for maintenance of the strain in the gut environment to exert its probiotic action. It has been described in *L. plantarum* strains that the surface-associated GAPDH is capable of interact to different matrices such as mucin and fibronectin suggesting that *L. plantarum* LA 318 adheres to human colonic mucin using GAPDH binding activity to colonize the human intestinal mucosa (Kinoshita et al., 2008). Consequently, the positive regulation of this enzyme could not only be associated to activation of the energy producing pathways but it could also be thought that the GAPDH isoenzymes that are up regulated could be associated to the cell wall and would play a key role in interaction with epithelial cells or host mucus. Nevertheless, bile or acid stress not always led to increased adhesion ability since results obtained with *L. delbrueckii* subsp. *lactis* 200 after growth in medium containing bile salt showed a reduced adhesion to epithelial cells, resulting in reduced persistence in the intestinal lumen and delayed capacity to activate the gut immune response (Burns et al., 2010; Burns, Reinheimer, & Vinderola, 2011).

Association to epithelial cells was ascribed to several factors. Denou et al. (2008) related long persistence of *L. johnsonii* NCC533 in the gut to the expression of genes involved in EPS biosynthesis meanwhile Ashida, Yanagihara, Shinoda, and Yamamoto (2011) described the presence of a surface protein (SlpA) in *L. acidophilus* that plays a key role in its attachment to Caco-2 cells.

In *Bifidobacterium*, it was shown that production of EPS is a response mechanism to bile stress (Ruas-Madiedo, Gueimonde, Arigoni, & de los Reyes Gavilan, C. G., & Margolles, A., 2009). Stress response of *L. paracasei* CIDCA 83123 led to a down regulation of galactose mutase, enzyme which rapidly catalyses the conversion of β-D-galactose to α-D-galactose (Beebe & Frey, 1998). Down-regulation of this enzyme would lead to an accumulation of β-D-galactose which could be a precursor of exopolysaccharides synthesis. The EPS constitutes a hydrophilic barrier that can protect the bacteria and could contribute to bacterial adhesion to surfaces, such as intestinal mucosa. Otherwise, the enzyme UTP glucose 1-phosphate uridil transferase which is involved in monosaccharide activation was up regulated in this strain being another factor which indicates that EPS production could be a response to GI stress. Though, no changes in colony phenotype of *L. paracasei* CIDCA 83123 stress-survivors were observed compared to non-stressed bacteria, EPS production per cell increased after gastrointestinal passage from 3.1 to 13.2 mg/10^8 CFU (unpublished data).

Adhesion of *L. paracasei* strains isolated from kefir to mucin and epithelial cells increases after gastrointestinal stress. This is the first report that study adhesion ability of *Lactobacillus* strains after sequential treatment that simulate gastric and intestinal conditions. Proteomic analysis of *L. paracasei* CIDCA 83123 revealed that proteins that were up regulated after gastrointestinal stress were those involved in carbohydrates metabolism focusing in modification of GAPDH isoforms and EPS biosynthesis. Considering this, the increased adhesion ability could be ascribed to both factors: increased EPS production and/or presence of proteins that switch between unrelated functions depending on the cell location.

These results demonstrate that *L. paracasei* strains studied isolated from kefir are potential candidates for the development of functional foods since gastrointestinal stress enhances their adhesion enabling the colonization of the human gut.

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