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Influence of inulin rich carbohydrates from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers on probiotic properties of *Lactobacillus* strains

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ABSTRACT

The effect of inulin extracted from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers on the growth and probiotic properties of different *Lactobacillus* strains was analyzed. It was demonstrated that *L. paracasei* BGP1 and *L. plantarum* CIDCA8327 strains were able to ferment inulin form Jerusalem artichoke tubers. The survival after simulated gastrointestinal conditions was improved in presence of inulin meanwhile the adhesion to intestinal epithelial cells capacity resulted strain- and inulin dose-dependent. Thus, the observed characteristics confer a potential application of this inulin rich carbohydrates extract as an ingredient that not only improves bacterial growth but also enhance its resistance to gastrointestinal conditions. These preliminary results regarding the probiotic-prebiotic interaction indicate that although inulin improved survival of the strains analyzed, the effect on adhesion ability depends on the probiotic studied.

1. Introduction

According to the definition, a functional food offers health benefits and contributes to decrease the risk of chronic diseases beyond its nutritional role. As a consequence of the increasing demand of functional foods by consumers, functional components such as probiotics and prebiotics have strategic importance in the industry for the development of new products (Al-Sheraji et al., 2013; Papadimitriou et al., 2015).

Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2006). The majority of microorganisms commonly employed as probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*. Probiotics for human consumption are of increasing interest due to the evidence of health benefits associated with their use, and they represent a significant growth area in the functional food industry (Guarner & Malagelada, 2003). The beneficial physiological effects attributed to probiotic bacteria are strain dependent, among them: reduction of pH in the gut and inhibition of human pathogens growth, production of bioactive peptides and vitamins, decrease of blood cholesterol level, antitumoral activity, stimulation of immune functions and improvement of calcium absorption (Pandey, Naik, & Vakil, 2015). In addition, probiotic microorganisms must be able, not only to arrive unaltered to the environment where they exert their beneficial effects on the host, but also to survive in it. Therefore, their resistance to human gastric and intestinal conditions constitutes an important parameter for the selection of probiotic microorganisms (Charteris, Kelly, Morelli, & Collins, 1998). Moreover, the capacity of probiotics to adhere to the intestinal epithelium would contribute to the colonization of the mucosal surfaces limiting the activity of pathogen microorganism and allowing the interaction with the immune cells (Muñoz-Provencio et al., 2009).

In the other hand, prebiotics are defined as substrates that are selectively utilized by host microorganisms conferring a health benefit (Gibson et al., 2017). Inulin and fructooligosaccharides (FOS) are the most commonly employed and studied prebiotics. Inulin consist in a fructan of linear chain, constituted by fructose molecules linked by $\beta(2 \rightarrow 1)$ bonds, with a terminal glucose unit linked by an $\alpha(1 \rightarrow 2)$ bond, with an average degree of polymerization (DP_n) typically from 10 to 12 units (Kays & Nottingham, 2007). Inulin stimulates the development and metabolic activity of a limited number of beneficial bacteria in the colon, particularly *Bifidobacteria* and *Lactobacilli*, and thus

Abbreviations: **CFU**, Colony forming units; **DPn**, Polymerization degree average; **FOS**, Fructooligosaccharides; **CI**, Commercial inulin from chicory; **IRC**, Inulin rich carbohydrates; **JAT**, Jerusalem artichoke tubers; **LAB**, Lactic acid bacteria; **OD**, Optical density; **PBS**, Phosphate buffered saline

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promotes a healthy microbial balance in the bowel, and also may provide the host defense against pathogen bacteria (Bosscher, Van Loo, & Franck, 2006). Besides the beneficial health effects of inulin, it offers technological advantages and is therefore widely used as an ingredient in functional foods (Shoaib et al., 2016).

Jerusalem artichoke (*Helianthus tuberosus* L.) is an economic crop cultivated in Europe, Asia and Australia (Nemeth & Izsaki, 2006). This crop is highly tolerant to adverse weather conditions and various plant diseases and also grows well in poor land. Jerusalem artichoke tubers store inulin as reserve carbohydrate (Saengthongpinit & Sajjaanantakul, 2005), so it has become an interesting crop at regional level with industrial potential. Commercially most of the inulin is produced from chicory and dahlia, however Jerusalem artichoke is also considered as good source for industrial production of a prebiotic ingredient (Flamm, Glinsmann, Kritchevsky, Prosky, & Roberfroid, 2001).

Probiotics microorganism could interact with prebiotics compounds and potentiate or modulate certain functional effects, and for these reason each particular combination should to be evaluated. The strain *Lactobacillus plantarum* CIDCA8327 is a potential probiotic with promising properties for the development of functional foods (Golowczyc et al., 2008). The commercial strains *Lactobacillus paracasei* BGP1, *Lactobacillus casei* BGP93 and *Lactobacillus acidophilus* ATCC314 are recognized probiotic microorganisms employed for functional food processing (Céspedes, Cárdenas, Staffolani, Ciappini, & Vinderola, 2013; Minervini et al., 2012; Tomaro-Duchesneau et al., 2014). Nowadays there is a growing interest of food industries for the development of products containing probiotic bacteria, and the use of prebiotics as food ingredients to enhance probiotic properties have been studied (Grimoud et al., 2010; Nazzaro, Fratianni, Nicolaus, Poli, & Orlando, 2012).

Scientific studies in relation to the interplay between probiotics and prebiotics and their effect at intestinal level, are a necessary step for the validation of functional claims and the development of functional food (Pandey et al., 2015; Vitali et al., 2012). The aim of this study was to evaluate the interaction of inulin obtained from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers as a component of culture medium as well as an ingredient for food where probiotics are intended to be incorporated simultaneously in order to identify suitable synergistic combinations. The results would allow finding new applications for the use of inulin from an alternative source.

2. Materials and methods

2.1. Raw material and chemical reagents

Jerusalem artichoke tubers (JAT), Bianca variety, grown and harvested in Córdoba, were kindly provided by INTA Manfredi (Córdoba, Argentina). Commercial inulin from chicory roots (CI) ($DP_n \geq 10$, carbohydrate content ≥99.5 g/100g, Orafti®GR, Belgium) was kindly donated by Saporiti S.A. (Argentina). Glucose was purchased from Merck. MRS medium was formulated as following (g L^{-1}): proteose peptone (10), beef extract (10), yeast extract (5), polysorbate 80 (1), ammonium citrate (2), sodium acetate (5), magnesium sulphate (0.1), manganese sulphate (0.05), dipotassium phosphate (2), cisteyne (0.5), for solid medium preparation agar-agar (15), pH adjusted to 6.8 ± 0.2. Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO, USA) was employed for cell culture. All the other chemicals used were of reagent grade.

2.2. Inulin rich carbohydrates extraction from Jerusalem artichoke tubers

Freeze-dried slices obtained from fresh tubers were ground in a domestic grinder (Braun, Mexico) to obtain a homogeneous powder. Inulin rich carbohydrates (IRC) were extracted in a batch-system. The extraction process was performed in a batch stirred system employing distilled water at 76 °C for 1.5 h, with a solid:solvent ratio of 1:16 (w/v) (Rubel, Iraporda, Novosad, & Manrique, 2018). The solution was separated from the slurry by successive filtrations through muslin cloth and paper filter. Then the extract was frozen and pulverized in a freeze-drier (LA-B3-C, Rificor, Argentina) until constant weight. Characterization of inulin rich carbohydrates from JAT was performed as described Rubel, Perez, Genovese, & Manrique (2014).

2.3. Bacterial strains and culture conditions

Commercial probiotic strains employed *Lactobacillus casei* BGP93, *Lactobacillus paracasei* BGP1 were kindly provided by CLERICI-SACCO (Cadorago, Italy) and *Lactobacillus acidophilus* ATCC314 purchased from American Type Culture Collection, was also used. The strain *Lactobacillus plantarum* CIDCA8327 belonging to CIDCA collection was isolated from kefir grains and previously identified and characterized as potential probiotic (Bosch et al., 2006; Golowzcyc et al., 2008; Hamet, Piermaria, & Abraham, 2015). All the strains were stored at −20 °C in MRS with skim milk and activated by incubation in MRS broth at 37 °C for 24–48 h.

2.4. Bacterial growth analysis

Lactobacillus strains were inoculated in MRS broth supplemented with commercial inulin (MRS_{CI}) and inulin rich carbohydrates from JAT (MRS_{IRC}) as carbon sources at 1% (w/v) at initial concentration of 10^5 UFC mL^{-1}. MRS carbohydrate-free basal medium (MRS_{Basal}) was employed as negative control growth; while MRS glucose at 1% (w/v) (MRS_{Glc}) was employed as positive control growth.

The pH was measured employing a pHmeter (HI 2211, HANNA, USA) and the optical density at 590 nm (OD_{590}) was determined in a spectrophotometer (UV-1800PC, Mapda Instruments, China), these measurements were done every 2 h until 8 h, and after 12, 24 and 48 h of incubation at 37 °C. Total viable bacterial after 24 h incubation at 37 °C were analyzed by viable plate count on MRS agar.

2.5. Lactic and acetic acid determination

Samples of spent culture supernatant were centrifuged (10 min, 5000 x g), deproteinized employing *o*-phosphoric acid 0.3% (v/v) and diluted in methanol as described (Jouany, 1982), for its analysis by gas chromatography coupled with a flame ionization detector (Trace GC-2000 ThermoQuest) using a ZB-FFAP column (Phenomenex, USA). Chromatographic conditions were: temperature gradient: 100–200 °C, ramp 5 °C min^{-1}, mobile phase: helium at 0.8 mL min^{-1}, injector temperature: 220 °C and detector temperature: 250 °C. Standard volatile free acid mix (CRM46975, Supelco, USA) was employed for the qualitative and quantitative characterization. The initial level of acetic acid detected in the non-fermented MRS broth was quantified and subtracted from the amounts calculated for the fermented samples. The amount of lactic acid in spent culture supernatant was determined enzymatically employing the commercial Kit Lactate (Wiener-Lab, Argentina) following the manufacturer instructions.

2.6. Thin layer chromatography

The non-fermented MRS_{IRC} and MRS_{CI} as well as the fermented culture supernatants were analyzed by thin layer chromatography (TLC). Samples of 2 mL were drawn at 0, 2, 4, 8, 12 and 24 h and centrifuged (10 min, 5000 x g) and the supernatant were stored at −20 °C until their TLC analysis. This assay was performed on Silica-gel 60 plates (Merck, Germany) using butanol:isopropanol:water:acetic acid (7:5:4:2) as mobile phase (Lingyun et al., 2007). Carbohydrates were visualized as spots by spraying a solution of *p*-amino benzoic acid (7 g L^{-1}) and *o*-phosphoric acid (30 g L^{-1}) in methanol (Zweig & Sherma, 1978) and heating at 100 °C for 10 min. Standards of fructose, sucrose, 1-kestose, nystose (Sigma-Aldrich, USA) 0.05% (w/v) were employed.

2.7. Bacterial tolerance to simulated gastrointestinal conditions

Lactobacillus strains grown in MRS_{Glc} were harvested after incubation at 37 °C during 24 h by centrifugation (10 min, 7000 x g) and resuspended in PBS. Then the bacteria were inoculated at a concentration 1×10^8 CFU mL^{-1} in a simulated gastric juice solution (NaCl 125 mM, KCl 7 mM, $NaHCO_3$ 45 mM, pH 2.5) with pepsin (2500 UI, SIGMA-Aldrich, USA) 3 g L^{-1}, previously sterilized by filtration (K18-230–KASVI, Brasil) and supplemented with CI or IRC from JAT at 1% (w/v). The suspensions were incubated during 1.5 h at 37 °C and washed twice with PBS. After that, they were centrifuged (10 min, 7000 x g). The bacterial pellets were resuspended in simulated intestinal fluid solution (NaCl 22 mM, KCl 3.2 mM, $NaHCO_3$ 7.6 mM, pancreatin (SIGMA-Aldrich, USA) 0.1% (w/v), bovine bile salts (SIGMA-Aldrich, USA) 0.15% (w/v), pH 8.0) previously sterilized by filtration (K18-230–KASVI, Brasil) and supplemented with CI or IRC from JAT 1% (w/v). Suspensions were incubated at 37 °C for 2.5 h. Bacterial viability was assayed as described Grimoud et al. (2010). The percentage of bacterial survival was calculated as follows:

$$\%\ survival = \frac{CFU/_{ml} after}{CFU/_{ml} before} x\ 100$$

Where CFU mL^{-1} represents cell viability at the beginning (*before*) and after incubation (*after*) in the simulated gastrointestinal conditions described above.

2.8. Bacterial adhesion to intestinal epithelial cells

The Caco-2/TC-7 clone (Chantret et al., 1994) established from the parental human enterocyte-like Caco-2 cell line, that model the mature enterocytes of the large intestine of were routinely grown in Dulbecco modified Eagle's minimal essential medium (DMEM) (Gibco BRL, USA) as described Zavala et al. (2016). Cells were seeded at a concentration $2.5x10^5$ cells $well^{-1}$ in 24-well tissue culture plates (Greiner Bio-one, USA). *Lactobacillus* strains grown in MRS_{Glc} were harvested after incubation at 37 °C during 24 h, and bacterial suspensions at concentration $1x10^8$ cell mL^{-1} in DMEM base with and without the supplementation with CI or IRC from JAT at 1 and 3% (w/v) were prepared. The cells seeded in 24-well tissue culture plates at confluence (10^6 cells $well^{-1}$) were washed twice with sterile PBS and were incubated with 0.5 mL of bacteria suspension (adjusted to 2×10^8 CFU mL^{-1}), for 1 h at 37 °C in a 5% CO_2 atmosphere. Then, the monolayers were washed three times with PBS, and lysed by addition of 1 mL of sterile distilled water. The number of viable bacteria adhered was assembled by colony counting in MRS agar (Zavala et al., 2016). The experiment was carried out in triplicate.

2.9. Statistical analysis

Results were expressed as mean ± standard deviation of at least three separate duplicate experiments. One way analysis of variance was performed. Mean differences were statistically tested using the LSD Fisher multiple comparison test conducted by the InfoStat® Software (*Version 2008*, Argentina). A p-value ≤ 0.05 indicated significant differences. Each assay was conducted at least in duplicate.

3. Results

3.1. Fermentation of inulin rich carbohydrates by probiotic Lactobacilli

The IRC were extracted from fresh JAT and the dry ingredient contained (% w/w) carbohydrates with DP_n 12 (85.6), proteins (9.9) and ash (4.4), with negligible levels of sucrose and fructose. The OD_{590} and acidification as a function of time as well as the count of viable bacteria obtained after 24 h for the *Lactobacillus* strains in MRS supplemented with the different carbon sources are presented in Fig. 1. *L. paracasei* BGP1 and *L. plantarum* CIDCA8327 strains could grow employing either IRC from JAT or CI, reaching OD_{590} that was not significantly different than the OD_{590} in MRS_{Glc} and significantly higher than in MRS_{Basal} (Fig. 1a). In concordance a significant decrease in pH in media with glucose, IRC and CI was evidenced for *L. paracasei* BGP1 and *L. plantarum* CIDCA8327 (Fig. 1b). The growth kinetics curves of *L. casei* BGP93 and *L. acidophilus* ATCC314 in MRS with both inulin as carbon sources were similar to that obtained in MRS_{Basal} (Fig. 1a), and both strains only presented a significant pH decrease in MRS_{Glc} (Fig. 1b).

In Fig. 1c it is showed that the growth of the strain *L. paracasei* BGP1 in MRS supplemented either with IRC or CI did not present significant differences with glucose, reaching values of 10.14 ± 0.15; 9.93 ± 0.55 and 10.00 ± 0.99 Log_{10} CFU mL^{-1}, respectively. The viable count of *L. plantarum* CIDCA8327 after 24 h in MRS_{IRC} did not present significant differences with MRS_{Glc}, reaching 9.34 ± 0.67 and 9.53 ± 0.39 Log_{10} CFU mL^{-1}, respectively. In both media the growth of *L. plantarum* CIDCA8327 was significantly higher than in MRS_{CI} (8.85 ± 0.37 Log_{10} CFU mL^{-1}). In contrast the viable count after 24 h for the strain *L. acidophilus* ATCC314 in MRS supplemented with CI or IRC did not present significant differences respect to the MRS_{Basal}. The growth of *L. casei* BGP93 in MRS_{IRC} was significantly higher than in MRS_{Basal}, however it did not reach the growth level as in MRS_{Glc}. These results indicate that *L. casei* BGP93 and *L. acidophilus* ATCC314 were not able to metabolize inulin under the conditions studied.

The TLC analysis of cultures supernatant of the strains that were able to growth in MRS_{IRC} is shown in Fig. 2. The strain *L. paracasei* BGP1 was able to consume fructans of diverse DP from the beginning, such as those presents in the MRS added with IRC from JAT. The strain *L. plantarum* CIDCA8327 showed initially a preferential consumption of DP < 3 fructans, and started to hydrolyze fructans of higher DP only after 8 h. A similar pattern was observed for the TLC analysis of the spent culture supernatant of both strains in MRS_{CI} medium (data not shown).

The concentration of lactic and acetic acid in spent culture supernatants after 24 h of incubation at 37 °C was analyzed for the strains *L. paracasei* BGP1 and *L. plantarum* CIDCA8327 and results are presented in Table 1. The concentration of lactic acid in MRS_{Glc}, MRC_{IRC} and MRS_{CI} media for the strain *L. paracasei* BGP1 resulted above 100 mmol L^{-1} and did not present significant differences among the media. For the strain *L. plantarum* CIDCA8327 the concentration of lactic acid was significantly higher in MRS_{Glc} than in the rest of the media analyzed, while in MRS_{Basal}, in which carbon sources from complex component of the medium are limited, resulted significantly lower than in MRS_{IRC} or MRS_{CI}, and between both media no significant differences were found. For both strains, the concentration of acetic acid was significantly higher in MRC_{IRC} than in the rest media studied.

3.2. Effect of carbohydrates on bacterial resistance to simulated gastrointestinal conditions

The survival of *Lactobacillus* strains after simulated gastrointestinal conditions was evaluated in presence of CI at 1 and 3% (w/v). This prebiotic compound significantly improved the *Lactobacilli* survival, and no significant differences in the results obtained for both concentrations were found (data not shown). For this reason, the concentration 1% (w/v) was employed for the comparative study. The results of the viability after simulated gastrointestinal conditions in absence (control) or in presence of CI and IRC are presented in Fig. 3. The presence of both inulin significantly increased the strains resistance to gastrointestinal conditions. The survival after simulated gastrointestinal treatment in control condition for *L. plantarum* CIDCA8727 (39.91 ± 9.02%) resulted significantly higher than the survival for *L. paracasei* BGP1 (21.97 ± 1.98%). Also, the presence of either CI or IRC improved survival of *L. plantarum* CIDCA8327 and no significant

differences between these compounds were detected. Finally, the survival of *L. paracasei* BGP1 was improved in presence of both inulin samples and resulted significantly higher with CI than IRC from JAT.

3.3. Effect of carbohydrates on bacterial adhesion to intestinal epithelial cells

Bacterial adhesion to Caco-2/TC-7 cells was tested in presence or absence of prebiotic compounds, and results are shown in Fig. 4. The strain *L. plantarum* CIDCA8327 was significantly more adherent than the strain *L. paracasei* BGP1, the percentages of adhesion in control conditions were 0.55 and 8.20%, respectively. The presence of CI or IRC from JAT either at 1 and 3% (w/v) did not affect the adhesion capacity of *L. paracasei* BGP1. In contrast, the presence of these compounds at a concentration of 3% (w/v) partially suppressed the adhesion capacity of *L. plantarum* CIDCA8327.

4. Discussion

The knowledge of the specific interaction of prebiotics and probiotics contribute to elucidate the potential symbiotic effects. The ability to employ inulin rich carbohydrates from JAT as carbon source for the growth of probiotic lactic acid bacteria was studied in the present work. The application of IRC from JAT as an ingredient of culture media for the production of starters or in fermented products containing probiotics requires the knowledge of its effect on both the bacterial growth and also the maintenance of the probiotic properties. The strains *L. acidophilus* ATCC314 and *L. casei* BGP93 were not able to ferment inulin since no significant growth of the strains neither acidification of the media was observed. These results indicated that these strains may not have the enzymatic machinery necessary for the inulin metabolism and for this reason, their probiotic properties in presence of these prebiotics were not analyzed. In contrast, the strain *L. paracasei* BGP1 acidified and grew up indistinctly in MRS media containing either glucose, IRC from JAT or CI as carbon source. The strain *L. plantarum* CIDCA8327 presented a similar growth in presence either of glucose or IRC from JAT, which were higher than with CI. Several authors reported the strain specific capacity of LAB to employ fructan from different sources (Zubaidah & Akhadiana, 2013; Pompei et al., 2007). Also, at intestinal level, the microbiota exert a cooperative effect that may contribute to the hydrolysis of long-chain fructans to be available for the consumption by microorganisms (Rossi et al., 2005). As could be detected in the TLC analysis, the strain *L. paracasei* BGP1 employed fructans with diverse DP within 8 h of incubation since a significant attenuation of the spots of all type of fructans was observed in this period. After 8 and 12 h of incubation, spots coinciding with the elution of the fructose and sucrose standards were detected indicating the hydrolysis of long-chain fructan polymers by *L. paracasei* BGP1 that were consumed after 24 h (no spot were observed). The strain *L. plantarum* CIDCA8327 employed short chain fructans (DP < 3) during a period of 8 h, and only after this time it started to hydrolyze and consume fructans with higher DP. The detection of a light spot coinciding with the elution of the fructose standard at 24 h for *L. plantarum* CIDCA8327 indicated the hydrolysis of long-chain fructan polymers (Fig. 2). In particular for the specie *L. plantarum,* Kleerebezem and Hugenholtz (2003) described the ability to adapt their metabolism for their survival in diverse environmental niches. Meanwhile, other LAB strains such as *L. gasseri* CECT5714 and *L. fermentum* CECT5716 used

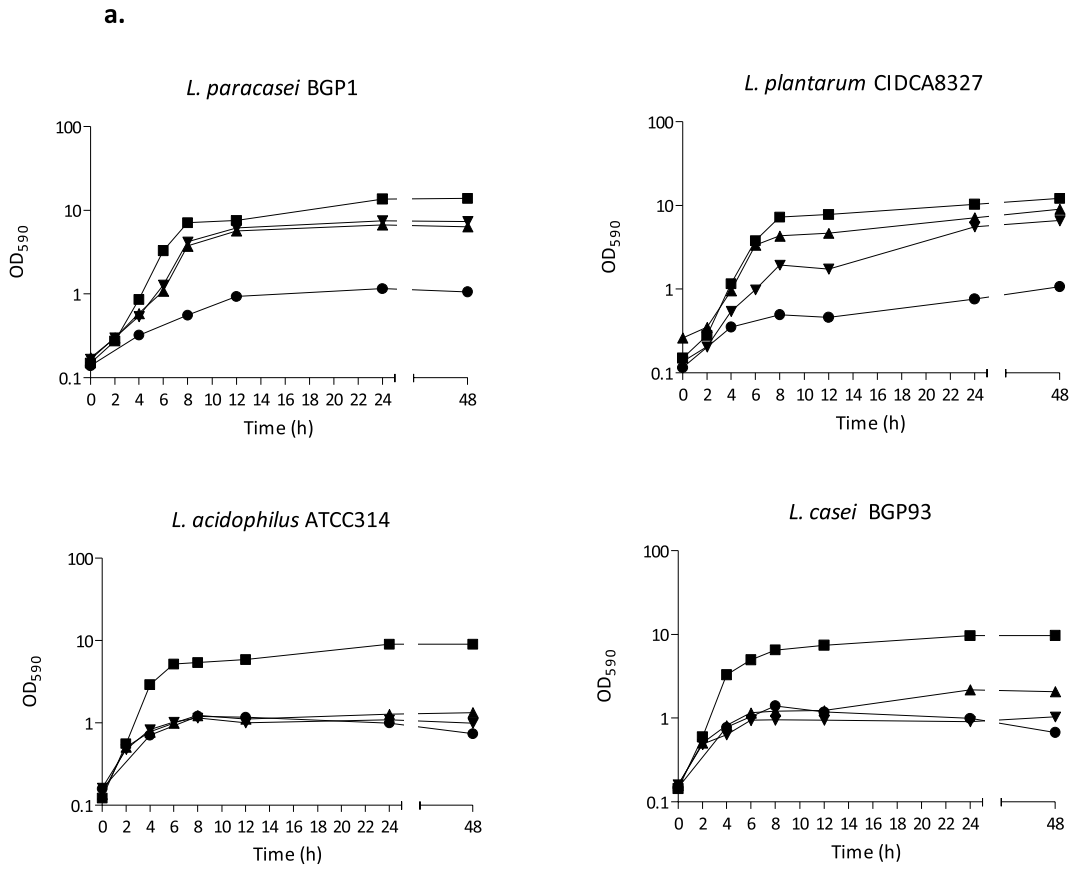

Fig. 1. *Lactobacillus* growth on MRS supplemented with different carbon sources. (a) Optical density at 590 nm (OD_{590}) vs. time (h) **(b)** pH vs. time (h) in MRS medium carbohydrate-free (MRS_{Basal}, ●) and supplemented at 1% w/v with glucose (MRS_{Glc}, ■), IRC from JAT (MRS_{IRC}, ▲) or commercial inulin (MRS_{CI},▼). **(c)** Total viable bacteria (Log_{10} CFU mL^{-1}) after incubation 24 h at 37 °C in MRS_{Basal} (□), MRS_{Glc} (▒), MRS_{IRC} (▓) and MRS_{CI} (■). Different letters indicate significant differences ($p < 0.05$) in the final count of each strain comparing the means obtained in MRS with the different carbon sources by ANOVA, followed by the LSD Fisher multiple comparison test.

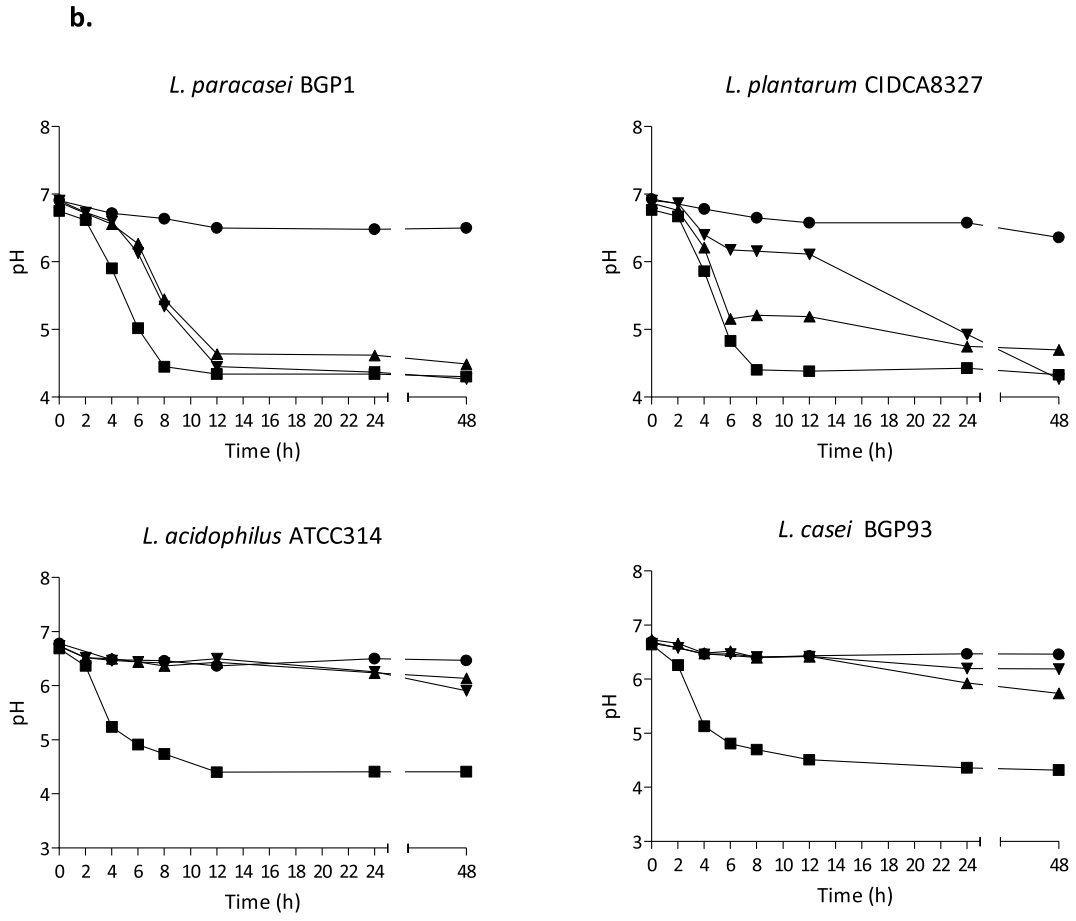

Fig. 1. (*continued*)

sucrose as carbon and energy source among others β(2-1) fructans, while they did not consumed fructans derived from inulin, independently of their DP_n (Bañuelos et al., 2008).

The differences in the levels of organic acid produced by the strains studied evidenced their metabolic differences. In the present work, it was observed that lactic acid was the main metabolic product of both *Lactobacillus* strains grown in MRS with different carbon sources. The concentration of lactic acid in the spent media for *L. paracasei* BGP1 were similar among carbon sources and resulted higher than the levels found in the spent culture supernatants of *L. plantarum* CIDCA8327. In another hand, levels of acetic acid in MRS_{IRC} for both strains were higher than levels obtained in the MRS with glucose or CI. Moreover

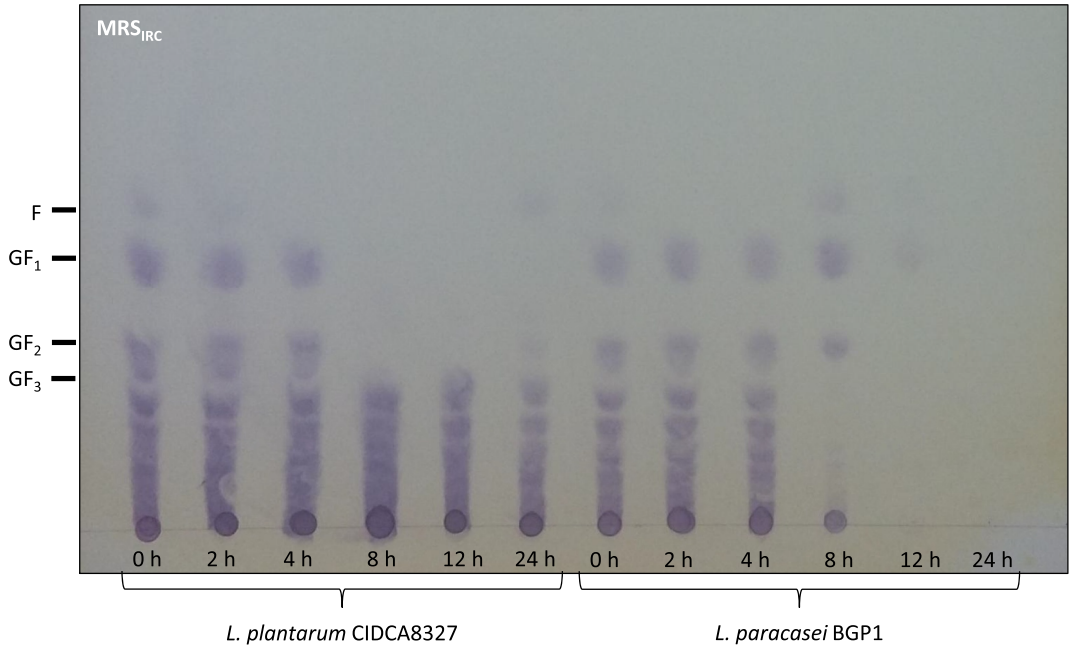

Fig. 2. Thin layer chromatography analysis. Culture supernatant of *Lactobacillus plantarum* CIDCA8327 and *Lactobacillus paracasei* BGP1 in MRS$_{IRC}$ incubated at 37 °C during different times (0, 2, 4, 8, 12 and 24 h). F: Fructose; GF$_1$: sucrose; GF$_2$: kestose; GF$_3$: Nystose.

Fukuda, Toh, Taylor, Ohno, & Hattori, 2012 reported that high levels of acetate are associated with specific modulation of immune response. The higher production of acetate by the strains analyzed employing IRC from JAT, respect to CI, makes this compound attractive to be employed as a functional ingredient. Nevertheless, as organic acids influence the organoleptic properties of a fermented product, this should be taken into consideration in the selection of the prebiotic in combination with a probiotic strain (Zalan et al., 2011).

The reason for the increased acetic acid production besides the carbon source is also the heterofermentative nature of the strains. The growth and organic acid production of different *Lactobacillus* strains on Jerusalem artichoke juice was evaluated by Zalan et al. (2011). These authors reported that lactic acid was the main acid produced (in concentration between 110 and 337 mmol L^{-1}) and the amount was strain specific. Makras, Van Acker, and De Vuyst (2005) also observed in inulin media that lactic acid was the main metabolic end-product of *L. paracasei* subsp. *paracasei* 8700:2, while for *L. paracasei* subsp. *paracasei* 2750 and *Lb. casei* subsp. *casei* 154 acetic acid was the main metabolic product. Higher amounts of acetic acid could be the result of different biochemical pathways, e.g. degradation of the produced lactic acid and/or it may be originated from the heterofermentative pathway (Zalan et al., 2011). Pompei et al. (2007) reported that the main products of fructan fermentation by LAB were acetic and lactic acid, and also lower concentrations of propionic and butyric acids were detected. Other authors reported that in presence of glucose *Lactobacillus plantarum subsp. plantarum* produced higher amounts of acetic acid than in presence of inulin, and that this substrate promoted the production of butyrate (Nazzaro et al., 2012). Also, Shalini, Abinaya, Saranya, and Antony (2017) reported high concentration of acetic acid produced by FOS and inulin fermentation by different LAB, while butyric acid was produced only by a strain of *L. plantarum* isolated from fermented *koozh*, indicating a strain specific fermentation ability.

Resistance to gastrointestinal conditions is an important requirement for probiotic microorganisms. In the present work the analysis of survival after the simulated gastrointestinal passage showed that the strain *L. plantarum* CIDCA8327 presented higher resistance than the commercial strain *L. paracasei* BGP1, indicating that this is a strain-dependent property, as was also suggested by Haller et al. (2001). It is important to notice that both strains, had received the same pre-treatments, since many authors have demonstrated that the tolerance to simulated gastric and intestinal juice is dependent on the growth conditions and carbon source, and is related with variation on the protein profiles (Nazzaro et al., 2012; Hernández-Hernández et al., 2012). In accordance with the results obtained in the present work, artichoke inulin addition to fruit juice improved the viability and survival after *in vitro* digestion of *L. plantarum* CECT220 respect to condition without inulin (Valero-Cases & Frutos, 2017). The exposure of probiotics to the acidic environment of the stomach could cause several damage to bacterial membranes that makes the cells more susceptible to bile action and enzymatic activity at the intestinal levels (Jin, Ho, Abdullah, & Jalaludin, 1998). Some previous studies have shown that the inclusion of prebiotic compounds improved the resistance to gastrointestinal conditions of different probiotics (Takemura et al., 2010). Moreover, Corcoran, Stanton, Fitzgerald, and Ross (2005) concluded that the survival of probiotic in acidic conditions occurred in the presence of sugars that they could metabolize efficiently via glycolysis, permitting optimal H^+ exclusion by ATPase. This leads to attribute the increase in the resistance to simulated gastrointestinal conditions of *L. paracasei* BGP1 and *L. plantarum* CIDCA8327 to the specific metabolism mechanisms of fructans.

In light of these findings, the IRC from JAT could be employed as a functional ingredient in combination with probiotics to improve survival of ingested probiotic bacteria during passage through the gut.

In this study, the adhesion to intestinal epithelial cells ability of *L. plantarum* CIDCA8327 resulted significantly higher than the adhesion capacity of *L. paracasei* BGP1, however it was observed that the adhesion capacity was partially suppressed by the presence of both inulin samples at the higher concentration analyzed, so it may reflex a dose-response effect. The adhesion capacity may be correlated with transient colonization of intestinal cells, which could be a factor for the potential benefits; however the relationship between adhesion capacity and the immune effect is strongly strain-dependent (Isolauri, Kirjavainen, & Salminen, 2002). Bacterial adhesion capacity may be related either to soluble adhesion mediating factors secreted into the spent culture supernatant or specific receptors of the bacterial surface (De-Palencia, López, Corbí, Peláez, & Requena, 2008; Haller et al., 2001). The partial suppression in adhesion of *L. plantarum* CIDCA8327 by inulin observed in a dose-response effect may be related to either structural interference

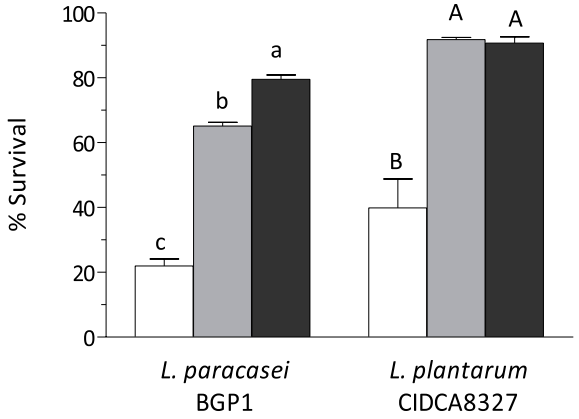

Fig. 3. Effect of inulin on bacterial resistance to simulated gastrointestinal conditions. Percentage of survival of *Lactobacillus* strains after simulated gastrointestinal treatment in absence (□) or presence of IRC from JAT (▩) and commercial inulin (■) at concentration 1% w/v. Results are expressed as mean ± SD of three independent experiments. Different letters indicate statistical differences for each strain ($p < 0.05$; ANOVA followed by the LSD Fisher multiple comparison test).

with the surface of the bacterial cell wall or with the enterocytes. Other author informed that FOS were slightly more effective compared to inulin at suppressing the adhesion to intestinal epithelial cells of a probiotic strain (Kim, Shin, & Park; 2015) and similar results were reported for oligosaccharides by Al-Tamimi, Abdelhay, and Rastall (2016). Therefore, the differences on anti-adhesion ability among the prebiotics could be presumably due to their structural differences.

In the present work it was demonstrated that the combination of *L. paracasei* BGP1 and *L. plantarum* CIDCA8327 with IRC from JAT may provide advantages promoting the growth and survival of the bacterial cultures; however the synergistic effects when incorporated to food matrices could not be generalized and should be carefully evaluated. The observed characteristics confer potential prebiotic properties of IRC from JAT, that should further evaluated *in vivo*, and suggest some possible applications in a symbiotic combination with the selected probiotics.

5. Conclusion

The results of this study represent evidence of interaction of inulin obtained from Jerusalem artichoke tubers with different *Lactobacillus* strains, which resulted comparable to that obtained with commercial inulin. It was demonstrated that *L. paracasei* BGP1 and *L. plantarum*

Table 1
Lactic and acetic acid in spent culture supernatants. Concentration of lactic and acetic acid (mmol/L) in culture supernatants obtained after incubation at 37 °C during 24 h of strains grown in MRS with addition of different carbon sources.

Strain	MRS Basal	MRS Glc	MRS IRC	MRS CI
	Lactic acid (mmol L^{-1})			
L. paracasei BGP1	7.8 ± 0.9 [a]	118.6 ± 1.0 [b]	121.3 ± 2.3 [b]	136.6 ± 1.5 [b]
L. plantarum CIDCA 8327	5.4 ± 0.5 [a]	84.4 ± 0.9 [c]	15.3 ± 0.7 [b]	18.5 ± 2.5 [b]
	Acetic acid (mmol L^{-1})			
L. paracasei BGP1	nd	32.3 ± 3.1 [a]	46.5 ± 5.3 [b]	28.3 ± 3.7 [a]
L. plantarum CIDCA 8327	3.7 ± 3.0 [a]	16.0 ± 11.0 [a]	44.6 ± 11.6 [b]	18.3 ± 5.3 [a]

The different letters indicate significant differences ($p < 0.05$) comparing the means obtained in the different media for each strain, analyzed by ANOVA followed by the Fisher LSD test.

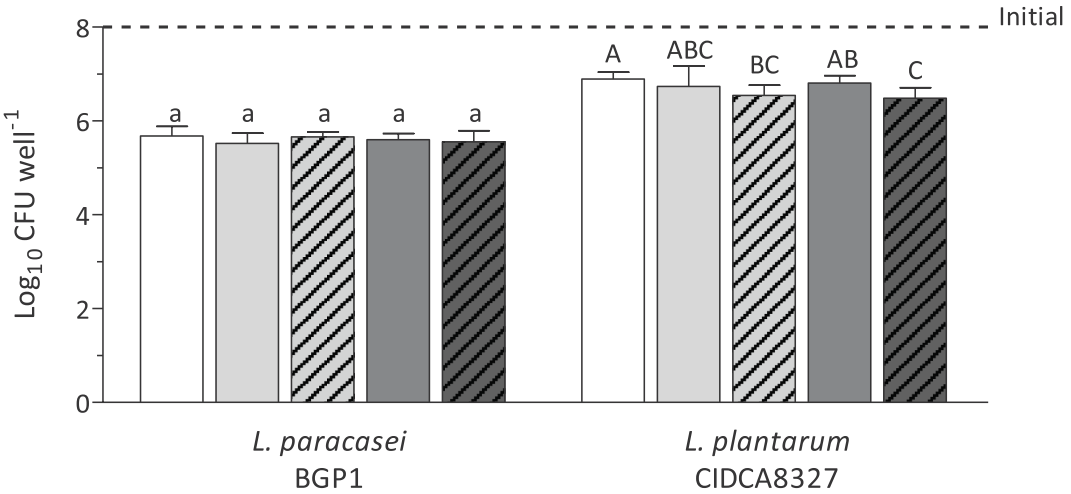

Fig. 4. Effect of inulin on bacterial capacity of adhesion to intestinal epithelial cells. Caco-2/TC7 cell adhesion capacity of *L. paracasei* BGP1 and *L. plantarum* CIDCA8327 (Log_{10} CFU $well^{-1}$) analyzed in the absence (Control, □) and presence of commercial inulin (CI, ▩) or IRC from JAT (IRC, ▩) at concentration 1% w/v (smooth bars) and 3% w/v (striped bars).
Results are expressed as mean ± SD of three independent experiments. Different letters indicate statistical differences for each strain ($p < 0.05$; ANOVA followed by the LSD Fisher multiple comparison test).

CIDCA8327 strains are able to ferment and to grow in MRS supplemented with inulin rich carbohydrates extracted from Jerusalem artichoke tubers. The probiotic properties such as tolerance to simulated gastrointestinal conditions was improved in presence of both inulin samples, meanwhile the adhesion to intestinal epithelial cells capacity resulted strain- and inulin dose-dependent. Therefore, more research work is needed for further specification and characterization of potential combinations for the development of novel functional foods.

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