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Whey permeate containing galacto-oligosaccharides as a medium for biomass production and spray drying of *Lactobacillus plantarum* CIDCA 83114

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ABSTRACT

Whey permeate (WP) is a low-cost waste product that can be used as a growth media of probiotic bacteria and as a source of galacto-oligosaccharides (GOS) being an excellent alternative to obtain probiotic biomass in a more economical way. The aim of this work was to evaluate the suitability of using WP and WP enriched with GOS (WP-GOS) as a culture broth and as a carrier for probiotic *Lactobacillus plantarum* CIDCA 83114 to obtain viable dehydrated bacteria using spray drying. This strain was able to grow satisfactorily in unsupplemented WP showing a similar behavior in WP and WP-GOS. It also performed well in spray drying. Viability of dehydrated lactobacilli was monitored throughout the storage of powders at 20 °C for 10 weeks. Survival during storage of *L. plantarum* grown and dehydrated in WP-GOS was significant higher than strain grown and dehydrated in WP at the end of storage time.

Strain grown in WP increased their tolerance to acid conditions and the presence of GOS increased significantly its survival at low pH environment in dehydrated condition. *L. plantarum* CIDCA 83114 grown and dehydrated in WP-GOS constitute a low-cost spray-dried preparation containing high concentration of viable bacteria with enhanced gastrointestinal passage resistance.

1. Introduction

Whey is a by-product of cheese making usually managed as a waste that, having a high biochemical oxygen demand (BOD), is costly to remove. Hence, the use of whey represents a very interesting option to give an added value to effluents (Marwaha & Kennedy, 1988). Whey proteins are generally separated from cheese whey by ultrafiltration, and employed as food additives or protein supplements. Therefore, the permeate remaining after whey protein recovery was mostly composed by lactose and salts. Whey permeate (WP) has multiple applications such as in bakery products, spice blends, snack foods, drink mixes, ice cream. Furthermore, due to its high content of lactose, it has been used as substrate which allows the growth of probiotic microorganisms (Golowczyc et al., 2013; Lavari, Páez, Cuatrin, Reinheimer, & Vinderola, 2014). This growth medium has the advantage of being more economical than traditional growth medium for lactobacilli. Often, this substrate is insufficient to obtain enough biomass of microorganisms and it is usually supplemented with other compounds such as yeast extract and vitamins (Cui, Wan, Liu, & Rajashekara, 2012; Hugenschmidt, Miescher Schwenninger, & Lacroix, 2011).

Probiotics were defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). Nowadays, probiotic microorganisms have great relevance worldwide because numerous studies have demonstrated several beneficial effects on human health (Shah, 2007). *Lactobacillus plantarum* CIDCA 83114 is a potential probiotic strain isolated from kefir grains. Numerous studies performed in our working group demonstrated interesting properties of the strain CIDCA 83114. In particular, the strain exhibited antimicrobial activity against *Salmonella enterica* serovar Typhimurium and *Shigella sonnei* (Golowczyc et al., 2008), decreased the adhesion of enterohaemorraghic *Escherichia coli* to Hep-2 cells (Hugo, Kakisu, De Antoni, & Pérez, 2008), protected cultured Hep-2 cells against *Shigella flexneri* and *S. sonnei* invasion (Kakisu, Bolla, Abraham, de Urraza, & De Antoni, 2013) and antagonized the cytotoxic effect of Shiga toxin produced by enterohaemorragic *E. coli* on Vero cells

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(Kakisu, Abraham, Tironi Farinati, Ibarra, & De Antoni, 2013).

Probiotic cultures for food applications are most frequently provided in frozen and dried forms and highly concentrated. Drying techniques to obtain dehydrated probiotic microorganisms in a viable state have proven to be useful. Freeze-drying has been the most widely used technique, but other drying methods such as spray drying, fluidized bed drying, vacuum drying and a combination of these techniques are used (Muller, Ross, Fitzgerald, & Stanton, 2009). Spray-drying is a lower cost technique and therefore, it is more convenient for producing large quantities of bacterial probiotic cultures (Corcoran, Ross, Fitzgerald, & Stanton, 2004; Desmond, Stanton, Fitzgerald, Collins, & Ross, 2001; Golowczyc, Silva, Abraham, De Antoni, & Teixeira, 2010). Previous studies have shown that microorganisms isolated from kefir grains maintained high viability values after spray-drying (Golowczyc et al., 2010; Golowczyc, Silva, Teixeira, De Antoni, & Abraham, 2011; Golowczyc, Gerez, Silva, Abraham, De Antoni & Teixeira, 2011) and freeze-drying procedures (Bolla, Serradell, de Urraza, & De Antoni, 2011). It is known that dehydration processes (if not correctly optimized) have a detrimental impact on the cellular integrity of probiotics and result in the loss of cellular viability and loss or changes in the probiotic properties. *L. plantarum* CIDCA 83114 has proved to be very resistant to dehydration processes such as spray drying when skim milk was used as a carrier (Golowczyc et al., 2010) and we have shown that some probiotic properties did not change significantly after this process (Golowczyc et al., 2011).

A nutritional supplement that combines probiotic and prebiotic is known as synbiotic. Most characterized prebiotics included oligosaccharides such as inulin, lactulose and fructo, gluco or galacto-oligosaccharides (Playne & Crittenden, 2009). Galacto-oligosaccharides (GOS) are prebiotics that have a beneficial effect on human health by promoting the growth of probiotic bacteria in the gut (Rastall, 2012). GOS are composed of a variable number of galactose units linked to a terminal glucose with different degrees of polymerization. Since more than seventy percent of WP is lactose is possible to generate *in situ* GOS by enzymatic synthesis using β-galatosidase from *Aspergillus oryzae* obtaining a new product enriched in GOS (Golowczyc et al., 2013). In this reaction, the enzyme catalyzed the lactose transgalactosylation to form new glycosidic bonds leading to the formation of GOS (Splechtna et al., 2006).

The use of WP as growth media of probiotic bacteria and as a source of GOS generate a synbiotic product from an economical substrate is an excellent alternative for the use of this second product of the cheese industry. Thus, the aim of this work was to evaluate the suitability of using WP and WP-GOS as a culture broth and as a carrier for probiotic *L. plantarum* CIDCA 83114 to obtain viable dehydrated bacteria using spray drying.

2. Material and methods

2.1. Bacterial strain

L. plantarum CIDCA 83114 were previously isolated from kefir grains, identified and characterized by Garrote, Abraham, and De Antoni (2001) and Delfederico et al. (2006). The strain was maintained frozen at −80 °C in 120 g L^{-1} non-fat milk solids. Microbial cells were reactivated in MRS broth (de Man, Rogosa and Sharpe) (Biokar, Beauvais, France) at 37 °C before conducting the experiments.

2.2. Growth conditions

Whey permeate (WP) was donated by Arla Foods Ingredients S.A. (Buenos Aires, Argentina). It was obtained by drying desprotenised sweet whey and contains approximately 80% (w/w), lactose, 6% (w/w) of ashes and 3% (w/w) of proteins as declared by the manufacturer. WP was rehydrated containing 20% (w/v) of solids and autoclaved for 15 min at 121 °C. WP was inoculated with *L. plantarum* CIDCA 83114 (1%) and incubated at 37 °C for 18 h. Culture aliquots were taken at different times of growth (0, 2, 4, 8, 16 and 18 h) to determine the viable cells by plate count in MRS agar. Growth in MRS broth under the same conditions was used for comparison.

2.3. Synthesis of galacto-oligosaccharides (GOS) from whey permeate

GOS synthesis was carried out as described by Golowczyc et al. (2013). Briefly, 40 g WP was mixed with 100 mM citrate-phosphate buffer pH 4.5 in order to obtain a reaction medium containing 40% (w/w) of solids which is the best concentration to obtain the maximum of GOS production. The previous suspension was heated over 95 °C to promote lactose dissolution and then the temperature was adjusted to 37 °C. Afterwards, 10 g enzyme solution were added to start the reaction of synthesis so that the enzyme dosage was 100 IU_T (international unit of transgalactosylation) per gram of lactose. The suspension was incubated at 37 °C for 1 h with constant stirring (150 rpm) and the reaction was stopped by boiling. A total of 27.4 g GOS/100 g lactose is produced in these conditions. The final product, WP-GOS, was neutralized, diluted to a concentration of 20% (w/v) and sterilized (121 °C, 15 min) prior to use it as growth media for the lactobacilli.

2.4. Spray-drying procedure

L. plantarum CIDCA 83114 was grown 18 h at 37 °C in WP or WP-GOS (prepared as described above), containing 20% (w/v) of solids. Microorganisms were dehydrated directly in the growth medium. A laboratory-scale spray-dryer (model B290 Büchi mini spray-dryer) was used to process samples at a constant air inlet temperature of 170 °C, an outlet temperature of 70–75 °C and a flux of 600 l/h. Results were compared with bacteria grown in MRS broth and dehydrated in MRS with maltodextrin 20% (w/v). Powder yield percentage was calculated as % weight fraction of the amount of fermented culture originally contained in the atomized liquid feed volume that could be recovered from the collecting vessel attached to the bottom of the cyclone. Powder present on the inside wall of the cyclone was not considered as being part of the yield.

2.5. Storage conditions

Spray-dried powders were stored during 70 days at 20 °C without fixing the relative humidity. The samples were taken out at different time intervals to determine their residual viability by plate counts. One gram of spray dried powder was rehydrated in 9 ml of salt solution (0.85% NaCl), homogenized for 1 min in a vortex mixer and maintained at room temperature for 30 min. Bacterial suspensions were serially diluted and plated on MRS agar. Bacterial counts were determined after 48 h incubation at 37 °C.

2.6. Water activity measurements

Water activity was measured after drying the samples using an Aqualab water activity instrument (Aqualab, Model Series 3 TE, USA). The equipment was calibrated using standard solutions provided by the manufacturer.

2.7. Acid and bile resistance

Cultures of the lactobacilli were washed with PBS and suspended in the same volume. Dehydrated lactobacilli were suspended in distilled water (1 g of powder in 2 ml of water) prior to the assay. To evaluate the acid resistance, samples were incubated in PBS at different pH ranges (from 2.3 to 2.9) for 2 h at 37 °C with agitation (100 rpm) and then they were incubated for 1 h in a bile solution (NaCl 1.28 g L^{-1}, KCl 0.24 g L^{-1}, $NaHCO_3$ 6.4 g L^{-1} and 0.5% of ox bile pH 7.2) at the same temperature and stirring conditions. Samples were mixed at a ratio of 1:9 to the acid PBS and then an equal volume of bile solution was added. Surviving bacteria were determined at the end of the acid treatment and at the end of the bile treatment by viable counts in MRS agar plates. Bile resistance was also evaluated by growing *Lactobacillus plantarun* in MRS agar with 5% (v/v) of ox bile (bile MRS agar). Dehydrated and non-dehydrated samples were processed as described before, a proper dilution of the samples was plated in bile MRS agar and MRS control. Viable counts were compared between the two media after incubation at 37 °C for 48 h.

2.8. Statistical analysis

All experiments were done on duplicate samples using three independent lactobacilli cultures. The relative differences were reproducible independently of the cultures used. Analysis of variance (ANOVA) of the viable counts corresponding to the different treatments was carried out using the statistical program Statgraphics Centurion XVII (Statistical Graphics Corp, USA). Comparison of means by Tukey methods were tested, and if $P < 0.05$ then the difference was considered statistically significant.

3. Results

3.1. Growth kinetics

L. plantarum 83114 was able to growth in unsupplemented WP showing a similar behavior in WP and WP-GOS. Lactobacilli achieved a maximum harvest of 5 10^8 CFU mL^{-1} (increased 1.3 log above the inoculum) and a generation time of 5.72 h^{-1} in average with a lag phase of 3 h. The presence of GOS had no effect on the kinetics parameters. MRS broth represents the optimum culture media for the *Lactobacillus* strains. In this case, *L. plantarum* exhibited a maximum harvest of 3.65 10^{10} CFU mL^{-1} (increased 3 log above the inoculum) a generation time of 0.85 h^{-1} and a lag phase of 1.5 h (Table 1).

3.2. Spray drying process

L plantarum 83114 was grown in MRS, WP and WP-GOS and spray dried in the same culture medium. The high counts of viable microorganisms obtained after spray-drying (above 10^8 per gram) indicate that is a thermo-resistant microorganism (Table 2). No significant differences between the survival of *L. plantarum* CIDCA 83114 grown and dehydrated in WP and WP-GOS were observed ($P > 0.05$). *L. plantarum* grown in MRS medium had a higher harvest than WP and WP-GOS (Table 1), thus the number of lactobacilli per gram after drying was significantly higher ($P > 0.05$) than *L. plantarum* CIDCA 83114 grown and dehydrated in WP and WP-GOS.

Powders obtained from WP were less adhesive (sticky) than powders obtained from MRS (with maltodextrin). Although, the concentration of solids was similar between the three culture media the powder yield from MRS was 10% and 15% lower than from WP and WP-GOS respectively. Water activity (a_w) of samples containing GOS was significantly lower ($P > 0.05$) than of samples without GOS (Table 2).

3.3. Survival during storage

Survival of dehydrated strain during storage at 20 °C was evaluated at different times without fixing relative humidity conditions (Fig. 1). Bacterial viability showed a decrease of 1.2 log unit in average during the first 4 weeks and there were no significant differences ($P < 0.05$) between the samples at this time. Viability drop was more noticeable from 7 to 10 weeks with 2.5 log drop in average. After 7 weeks of storage period, no significant differences were observed between survival of lactobacilli grown and dehydrated in WP and WP-GOS. However, at this time, viability of *L. plantarum* grown and dehydrated in MRS was significant lower ($P < 0.05$) compared to *L. plantarum* grown and dehydrated in WP and WP-GOS. At the end of the storage period (10 weeks), viability in MRS samples fell almost to an undetectable value with 5 logs drop. At this time, WP and WP-GOS samples showed a less severe loss of viability with 2.6 and 1.9 logs drop respectively. Viability of *L. plantarum* grown and dehydrated in WP-GOS was significantly higher ($P < 0.05$) than *L. plantarum* grown and dehydrated in WP at the end of storage time.

3.4. Acid and bile resistance

L plantarum CIDCA 83114 was extremely resistant to bile regardless of the culture media employed for its growth. In fact, there were no differences between bacterial counts of the non-dehydrated and dehydrated samples from WP, WP-GOS or MRS, growing in presence or absence of 5% of ox bile (Fig. 2).

Survival of dehydrated and non-dehydrated lactobacilli to acid and bile presence (0.5%) are shown in Fig. 3 a and b. Strain CIDCA 83114 was very resistant to acid conditions above pH 3 (data non shown) so we evaluated three ranges of pHs below this value (2.3 ± 0.1; 2.6 ± 0.1 and 2.9 ± 0.1). Dehydrated and non-dehydrated MRS samples were the less resistant to the acid and bile treatment with high loss of viability in all the pHs assayed. WP samples showed a higher viability than MRS samples with the exception of those at pH 2.3 which had an important drop of viability similar to than MRS samples. Dehydrated WP samples were more sensitive to the acid and bile treatment than WP non-dehydrated ones, except at pH 2.3 in which both exhibited 5 logs of decrease in the viable counts. Viability of the lactobacilli in WP-GOS was similar between dehydrated and non-dehydrated samples in all the pHs assayed.

Table 1
Kinetic parameters of *L. plantarum* CIDCA 83114 grown in MRS, whey permeate (WP) and in whey permeate containing GOS (WP-GOS). Kinetic was determined by viable counts.

Culture broth	Lag time (h)	Growth rate (μ; h^{-1})	Generation time (g; h)	Maximum harvest (CFU mL^{-1})
MRS	1.5	0.819	0.85	$3.65\ 10^{10} \pm 9.19\ 10^{9}$
WP	3	0.110	6.30	$4.00\ 10^{8} \pm 6.36\ 10^{7}$
WP-GOS	3	0.160	4.30	$4.75\ 10^{8} \pm 7.07\ 10^{7}$

Table 2
Survival, water activity (a_w) and powder yield of L. plantarum CIDCA 83114 grown in MRS, whey permeate (WP) and in whey permeate containing GOS (WP-GOS) after spray-drying. Different letters indicate significant differences ($P < 0.05$).

Culture media	Bacterial counts before spray drying (log CFU mL^{-1})	Bacterial counts after spray drying (log CFU g^{-1})	a_w	Yield (%)
MRS	9.28 ± 0.15	8.74 ± 0.57*	0.368 ± 0.069[a]	9.60
WP	8.28 ± 0.07	8.15 ± 0.07	0.302 ± 0.009[a]	10.50
WP-GOS	7.99 ± 0.01	7.71 ± 0.01	0.252 ± 0.005[b]	11.22

* Value corresponds to lactobacilli grown in MRS medium and dehydrated in MRS added with maltodextrin 20% (w/v).

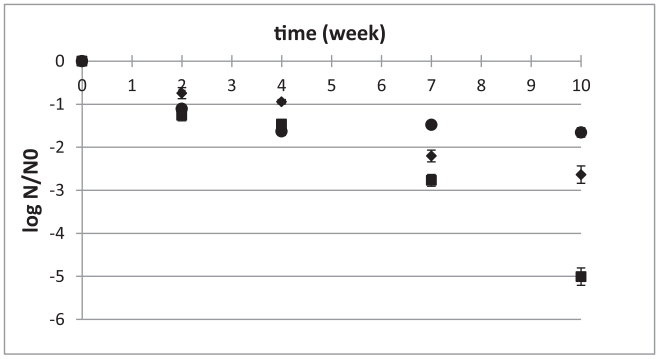

Fig. 1. Storage stability of dehydrated *L. plantarum* CIDCA 83114. The strain was grown and dehydrated on different substrates. Results were expressed as logarithmic values of relative survival fraction (log N/N_0) as a function of storage time at 20 °C: (●) lactobacilli grown and dehydrated in WP-GOS, (◆) lactobacilli grown and dehydrated in WP, (■) lactobacilli grown in MRS broth and dehydrated in MRS broth with 20% maltodextrin.

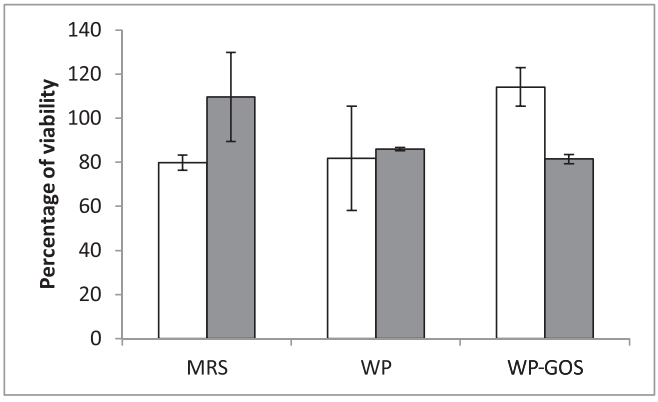

Fig. 2. Bile tolerance of dehydrated and non-dehydrated *L. plantarum* CIDCA 83114 in MRS agar with 5% (v/v) of ox bile. The strain was grown and dehydrated on different substrates (WP, WP-GOS and MRS). Viability was expressed as percentage respecting control. Control represents viable bacteria grown in MRS without bile and was considered as 100%. White bars represent the percentage of viable bacteria obtained from non-dehydrated samples. Grey bars represent the percentage of viable bacteria obtained from dehydrated samples.

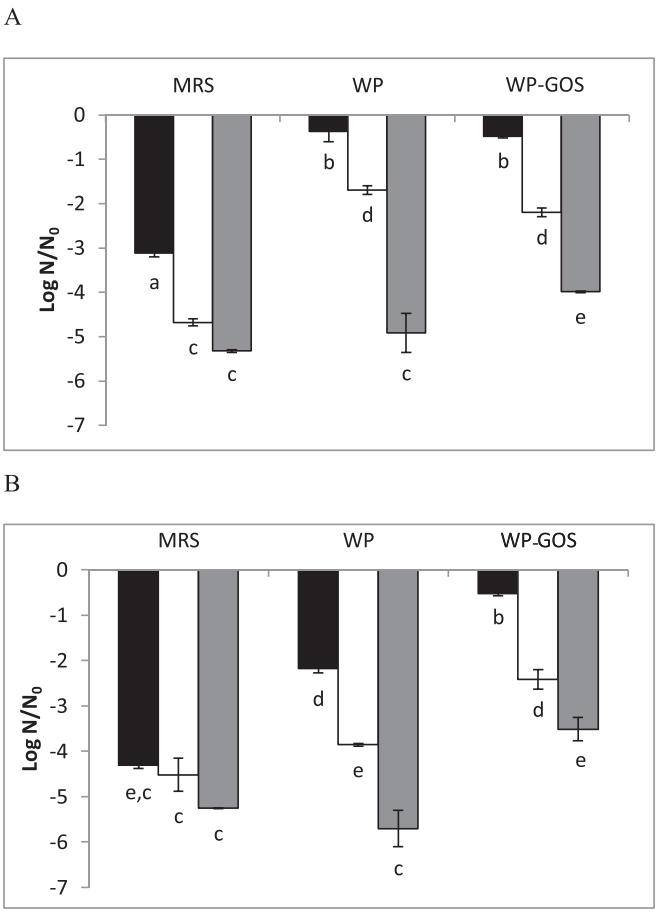

Fig. 3. Relative survival fraction (log N/N_0) of L. plantarum CIDCA 83114 after incubation with acid and bile solutions. Samples were incubated 2 h in acid PBS at different pHs and then 1 h with a solution containing 0.5% of ox bile. Results expressed viability of the lactobacilli after the whole treatment. (A) Non-dehydrated (fresh culture) *L. plantarum* grown in MRS, WP and WP-GOS. (B) Dehydrated *L. plantarum* grown in MRS, WP (WP-GOS). Color bars indicate different pHs of the PBS solution. Black bars pH 2.9 ± 0.1, white bars pH 2.6 ± 0.1 and grey bars pH 2.3 ± 0.1. Different letters indicate significant differences ($P < 0.05$).

4. Discussion

In this work we use WP and WP-GOS as growth medium of *L. plantarum* CIDCA 83114 strain isolated from kefir grain and as a carrier of spray drying, focusing on making the production of dried probiotic easier. We also study the influence of this process in cell viability and resistance to acid and bile. We observed that this strain exhibited similar growth kinetics in WP and GOS-WP. The addition of GOS as an extra hydrocarbon source had no effect in the kinetic parameters, probably due to the excess of lactose of the medium. WP and WP-GOS are poor culture media for lactobacilli comparing with MRS. It has been shown that to obtain high bacterial yields from whey permeate it is necessary to add expensive sources of proteins as yeast extract (Hugenschmidt et al., 2011; Lavari et al., 2014). However, in our work WP formulations (without supplements) showed a maximum harvest of approximately 5 10^8 CFU mL^{-1}, which is an acceptable concentration of viable bacteria for a probiotic product (Kosin & Rakshit, 2006; Saarela, Gunnar, Rangne, Jaana, & Mattila-Sandholm, 2000).

L. plantarum CIDCA 83114 showed high viability after spray-drying regardless the growth medium used. In a previous work, this strain grown in MRS and dehydrated in skim milk, showed the highest survival rate among other kefir strains during spray drying and storage (Golowczyc et al., 2010). *L. plantarum* grown in MRS and dehydrated in MRS with maltodextrin (added to have the same

amount of solids containing WP and WP-GOS) was used to compare with the other alternative media. It is known that acidity of the growth medium could decrease bacterial survival in a dehydration process (Golowczyc et al., 2013). In our study, the acids generated during lactobacilli growth were present in the drying medium and may cause unfavorable conditions for lactobacilli survival. In spite of the acidic environment, high values of viable *L. plantarum* cells were obtained (about 10^8 per gram) after the drying process. GOS had demonstrated to enhance bacterial tolerance in dehydration processes (Tymczyszyn, Gerbino, Illanes, & Gómez-Zavaglia, 2011; Tymczyszyn et al., 2012). In this report, no significant difference ($P < 0.05$) in survival during the drying process in lactobacilli grown and dehydrated in WP with and without GOS was observed. The high thermotolerance of the strain probably masked this protective effect.

Dehydrated strain CIDCA 83114 from WP, WP-GOS and MRS were stored at 20 °C without fixing relative humidity conditions. It is known that the storage at temperatures above 4 °C and relative humidity above 11% decrease the loss of viability in spray-dried powders containing bacteria (Golowczyc et al., 2010; Golowczyc et al., 2011). However, we selected these unfavorable conditions because they are the most economical to store probiotic products at commercial level. In general, the storage of *L. plantarum* strains at 25 °C and 33% of RH generates high rates of specific degradation of dried powders leading viability to undectectable levels between 60 and 80 days (Lapsiri, Bhandari, & Wanchaitanawong, 2013). Viability of *L. plantarum* dehydrated in MRS (with maltodextrin) confirmed these results with a dramatic loss of viability between 7 weeks and 10 weeks of storage. Interestingly, WP and WP-GOS samples showed a significant higher viability than MRS samples in this time period, exhibiting a drop of 2.25 logs viability in average. Ananta, Volkert, and Knorr (2005) reported that the incorporation of commercial GOS in the carrier (skim milk) did not exert any adverse effect on bacterial survival upon spray drying. However, the same author reported that storage stability at 25 °C was impaired by partial substitution of skim milk by prebiotic substances. Golowczyc et al. (2011) reported that the presence of prebiotic FOS increased survival of spray dried kefir strains stored at low concentrations relative humidity. In this work, we have observed a significant increase in survival of dehydrated *L. plantarum* in the presence of GOS when WP was used as a carrier.

It is known that higher water activity induced lower stability of microorganism in powder form (Abe, Miyauchi, Uchijima, Yaeshima, & Iwatsukie, 2009; Golowczyc et al., 2010; Poddar et al., 2014). In our work, the lowest a_w values corresponded to powder contained *L. plantarum* grown and dehydrated in WP-GOS (0.252). According to was previously mentioned, this powder showed the highest viability after 10 weeks of storage. Therefore, survival of these cultures during storage could be improved under controlled a_w (Poddar et al., 2014).

Strain CIDCA 83114 is highly resistant to bile, being able to grow in presence of 5% of ox bile. Interestingly, this capacity was not affected neither by the culture media employed in the growth of the lactobacilli nor by the spray drying process. Acidity was the most important factor that influenced lactobacilli viability being the strain mainly sensible to pH ranges of 2.3 ± 0.1. Overall, cultures from WP and WP-GOS presented a higher resistance to low pHs than cultures from MRS. Dehydrated bacteria, which suffered a previous hot stress, exhibited lower survival values probably due to damage caused to the cell membrane during the process. In this sense, MRS and WP samples showed a significant decrease of viability between dehydrated and non-dehydrated lactobacilli in pHs 2.9 and 2.6. The presence of GOS abolished the difference between dehydrated and non-dehydrated lactobacilli, increasing the survival of the dehydrated strain in WP-GOS samples at low pH.

Adverse conditions or stresses during microbial growth can lead to enhanced tolerance responses (Peighambardoust, Tafti, & Hesari, 2011). WP constitutes a poor culture media for lactobacilli. Its composition revealed more than more than 70% of lactose which constitutes almost the only carbon source for the growth of the lactobacilli. Due to the necessity of the dehydration process and GOS synthesis, our formulations have a high content of solids (20% w/v) creating an osmotically adverse environment. We hypothesized that these stress factors could enhance acid tolerance of strain CIDCA 83114 growing in WP. Moreover proteomics studies of *L. plantarum* 423 revealed that the main mechanism involved in an acid challenge is to utilize different carbon sources to supply cell energy and to produce basic compounds (Heunis, Deane, Smit, & Dicks Leon, 2014). It is possible that growth in WP rich in lactose allows the derepression of some of this alternative metabolic pathway giving an advantage against the acid stress.

GOS is a well-known prebiotic that stimulates the growing of bifidobacteria and lactobacilli at the intestinal level (Boehm & Stahl, 2003). In addition it was shown that growth of some *Lactobacillus* strains with GOS as the main carbon source could help to increase their resistance to gastrointestinal conditions (Hernandez–Hernandez et al., 2012). In our study, GOS presence improves bacterial survival to acid conditions mainly in dehydrated lactobacilli. Therefore, *L. plantarum* 83114 grown and dehydrated in WP-GOS combines probiotic bacteria with enhance resistance to low pH and prebiotic.

The use of economic growth media to obtain probiotic biomass is a great interest at industrial level as a way to reduce costs and extend their incorporation into different foods or additives. In this sense, by-products as whey and whey permeate constitute an alternative for the production of probiotic bacteria. Spray drying is a low-cost alternative technique in order to produce dehydrated biomass of probiotics to be applied in functional foods (Meng, Stanton, Fitzgerald, Daly, & Ross, 2008). However, the application of this drying technology, affects bacterial viability and activity since cells can suffer from a variety of stresses including heat, osmotic and oxidative stress. In order to apply this methodology successfully, it is necessary to maintain a high viability of the probiotic strain during drying, storage and passage through the gastrointestinal tract. In our work, we demonstrated that *L. plantarum* CIDCA 83114 was able to grow in unsupplemented WP maintaining high viability after the drying process. When the strain was grown in WP-GOS significantly improved their survival during storage even in unfavorable environment. The growth of the strain in WP increased its tolerance to acid conditions and the presence of GOS increased significantly its survival at low pHs in dehydrated condition. In conclusion, *L. plantarum* CIDCA 83114 grown and dehydrated in WP-GOS constitutes a low-cost spray-dried preparation containing high concentration of viable bacteria with enhanced resistance to the passage through the gastrointestinal tract.

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