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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.