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Spray-dried adjunct cultures of autochthonous non-starter lactic acid bacteria

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ABSTRACT

Spray-drying of lactic cultures provides direct-to-vat starters, which facilitate their commercialization and use. However, this process may alter the metabolic activity and deteriorate technological features. In this work, we assessed the influence of spray-drying on the survival and aroma production of two strains of mesophilic lactobacilli: *Lactobacillus paracasei* 90 and *Lactobacillus plantarum* 91, which have already been characterized as good adjunct cultures. The spray-drying was carried out using a laboratory scale spray and the dried cultures were monitored during the storage for the survival rate. The dried cultures were applied to two cheese models: sterile cheese extract and miniature soft cheese. The influence on the carbohydrate metabolism and the production of organic acids and volatile compounds was determined. Both strains retained high levels of viable counts in the powder after drying and during the storage at 5 °C for twelve months. In addition, they also remained at high level in both cheese models during incubation or ripening. Similar profiles of carbohydrate fermentation and bioformation of volatile compounds were observed in the cheese extracts for each of the strains when tested as both fresh and dried cultures. In addition, the ability of *Lb. paracasei* 90 to increase the production of acetoin and diacetyl remarkably in cheese models was also confirmed for the spray-dried culture.

1. Introduction

Non-starter lactic acid bacteria (NSLAB) are important components of cheese microbiota because they represent an adventitious and uncontrolled group of lactic acid bacteria that can attain high levels after a few weeks during ripening and prevail or remain stable for several months (Beresford et al., 2001; Crow et al., 2001; Gobbetti et al., 2015; Porcellato and Skeie, 2016). The addition of selected strains of NSLAB to cheese milk is an interesting approach in producing desirable changes in cheese, such as controlling the adventitious flora and improving the flavor or texture (Gobbetti et al., 2015; Johnston et al., 2010). Many strains, mainly mesophilic lactobacilli, isolated from good-quality cheeses, have shown positive effects of their action on cheese quality (Ciocia et al., 2013; Crow et al., 2001; Di Cagno et al., 2012; Randazzo et al., 2008; Settanni and Moschetti, 2010; Settanni et al., 2011; Wouters et al., 2002). However, the commercialization of such NSLAB strains for dairy industry requires a stable and convenient formulation with high cell concentration and ensuring a long-term viability and the preservation of metabolic activity during the storage (Parente and Cogan, 2004). There are very few investigations on the production of adjunct cultures in an appropriate form that can be applied in cheesemaking from the NSLAB strains isolated and characterized (Gobbetti et al., 2015; To and Etzel, 1997).

Lactic cultures are usually preserved by freeze-drying, freezing, or spray-drying. The freeze-dried cultures can be traded and stored at room temperature, although preservation at 4 °C or − 20 °C prolongs the survival and improves metabolic activity (Parente and Cogan, 2004). Freezing at a very low temperature (− 80 or − 196 °C) is the best way to preserve the viability and activity of bacteria; however, the handling of frozen cultures is not practical. In recent years, the interest in spray-drying as a preservation method for bacteria has grown. This technology offers advantages such as high production rates, low operating costs, single-unit processing for particle formation and drying, readily available machinery, and the ease of scaling up (Ghandi et al., 2012; Santivarangkna et al., 2007). Thus the industrial spray dryers, which are used in medium to big-size dairy industries for

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the production of dried skim milk and food ingredients can be exploited to produce spray-dried adjunct cultures (Lavari et al., 2015). However, the survival of bacteria to spray-drying process depends on many factors, such as strain, growth phase, growth conditions, and the parameters of spray-drying. In particular, the spray-drying process involves different parameters, such as outlet and inlet air temperatures, air flow rate, product feed rate, and atomized drop sizes, which could influence the microbial survival (Santivarangkna et al., 2007). Even if the initial attempts to use spray-drying for bacteria were unsuccessful (low survival after spray-drying and during storage, delay in lactic acid production, and the difficulty in rehydration of the product) (Johnson and Etzel, 1995; Teixeira et al., 1995; To and Etzel, 1997), the current technology allows an improved control of the conditions of the spray, and excellent preservation rates have been found for several strains of lactic acid bacteria (Silva et al., 2011). There are some reports related to the production of dehydrated probiotic cultures from different strains of bifidobacteria and lactobacilli, and in these reports the maintenance of the survival and the probiotic functionality have been given the prime importance (Gardiner et al., 2000; Golowczyc et al., 2011; Páez et al., 2012). In addition, there are few studies wherein the spray-drying was applied as a method to prepare attenuated bacteria of *Lb. helveticus* and *Lb. casei* to accelerate Cheddar cheese ripening (Johnson et al., 1995; Madkor et al., 2000). The addition of these adjunct cultures in the cheese led to the enhancement of flavor and reduced the off-flavor, above all when a higher outlet air temperature was applied during the spray-drying process (Johnson et al., 1995). To the best of our knowledge, there are no reports determining the effect of the addition of spray-dried adjunct cultures from autochthonous strains on the volatile compounds production in cheese.

On the other hand, most of the studies about the addition of lactobacilli strains as adjunct cultures in cheese generally considered the assay of a unique dose, while few other studies evaluated different doses of these cultures with an aim to select the optimal dose to produce a desirable effect on cheese (Carunchia Whetstine et al., 2006; Van Hoorde et al., 2010).

In previous investigations, we isolated and characterized two mesophilic lactobacilli strains: *Lactobacillus paracasei* 90 and *Lactobacillus plantarum* 91. These autochthonous NSLAB obtained from good quality semi-hard cheese showed desirable properties as adjunct cultures when they were tested as fresh cultures in Pategrás and Cremoso cheese varieties (semi-hard and soft cheese, respectively). In these cheese varieties, both strains led to beneficial changes during ripening: increase of peptidolysis, enhancement of flavor and control of adventitious microflora (Milesi et al., 2009, 2010).

The aim of this work was to obtain spray-dried adjunct cultures from *Lactobacillus paracasei* 90 and *Lactobacillus plantarum* 91, and to assess their ability to produce flavor-related biochemical events in two cheese models. The performance of the dehydrated versus fresh cultures was compared and the best dose of dehydrated *Lb. paracasei* 90 was assessed.

2. Materials and methods

2.1. Strains and culture conditions

Lb. paracasei 90 and *Lb. plantarum* 91 were obtained from the culture collection of Instituto de Lactología Industrial (INLAIN, Santa Fe, Argentina). The technological and biochemical features of both strains have been documented previously (Milesi et al., 2008; Peralta et al., 2014, 2016a, 2016b). The stock cultures of the strains were stored at − 80 °C in MRS broth (Biokar Diagnostics, Beauvais, France) with the addition of glycerol 15% (v/v) as a cryopreservative. Each strain was revived (2% v/v) twice in the broth overnight at 37 °C prior to the use.

2.2. Spray-drying of lactobacilli strains

Each lactobacilli strain was grown in MRS broth (Biokar Diagnostics) at 37 °C for 16–18 h. Then, the cultures were centrifuged at 6000 × *g* for 15 min at 5 °C, the pellets were washed twice with 50 mM potassium phosphate buffer (pH 7), and then resuspended in 20% (w/v) skim milk. The cell suspensions were spray-dried in a laboratory scale spray dryer (Buchi mini spray dryer model B290, Flawil, Switzerland) with a constant inlet air temperature of 140 °C, an outlet temperature of 82 °C, and a flux of 600 L/h. The cell suspensions were atomized and sprayed into the drying chamber using a two-fluid nozzle. The drying process was almost instantaneous and the residence time was negligible. The powders were vacuum sealed in individual packs. The residual moisture content (% w/w) of the powders was determined by drying at 101 ± 1 °C until a constant weight was achieved (FIL-IDF, 1993b). The cell counts of the lactobacilli in the suspensions before spray-drying, in the powders immediately after the process, and after 8 and 12 months of storage at 5 °C were determined on MRS agar (48 h of incubation at 37 °C). The powders were reconstituted in 0.1% casein peptone water to the original liquid volume (i.e., 0.2 g powder/mL) for the cell count determination.

2.3. Cheese models

The ability of the strains to produce flavor compounds was assayed for both fresh and dried cultures in two cheese models. *Lb. plantarum* 91 and *Lb. paracasei* 90 were tested in a sterile extract of soft cheese (Peralta et al., 2016b), while *Lb. paracasei* 90 was also assessed in miniature soft cheese (Milesi et al., 2010).

2.3.1. Sterile cheese extract

Soft cheeses were manufactured to obtain the extract; cheesemaking was made according to Milesi et al. (2009). After 20 days of ripening at 5 °C, the cheeses were grated and homogenized with distilled water (1:1), and the resultant slurry was centrifuged (17,000 × *g*, 15 min, 10 °C). The soluble fraction was extracted, and standardized with sodium chloride at 1.5% (w/v), filtrated through PVDF membranes of 0.4 μm (Millipore, Sao Paulo, Brazil) and heated 30 min at 70 °C. The initial pH value of the extracts was noted to be 5.20.

The experimental extracts were individually inoculated with each strain in order to reach an initial concentration of 10^6 CFU/mL and then incubated at 37 °C for 48 h. The cultures were added in three different forms: as fresh (F), spray-dried (D), and reactivated spray-dried (R) cultures. An aliquot of a fresh overnight culture of the strains revived on MRS (after two successive overnight incubations) was inoculated in F extracts. For D extracts, a quantity of the spray-dried powder was added directly to the extract, and for R extracts, the spray-dried powder (0.5 g) was reactivated by two successive overnight incubations in MRS broth. The non-inoculated extracts served as the controls. Microbiological counts, pH, carbohydrates, organic acids, and volatile compounds were determined in the extracts during the incubation. All of the extracts were prepared in duplicate using two independent cultures of the strains.

2.3.2. Miniature soft cheese

The spray-dried culture of *Lactobacillus paracasei* 90, which demonstrated the best performance for the production of cheese flavor compounds in the cheese extract, was tested as adjunct culture in miniature soft cheese. Four types of cheese were manufactured: one control cheese (C) containing *Streptococcus thermophilus* as the primary starter, and three experimental cheeses (E) with the addition of the same primary starter and *Lactobacillus paracasei* 90 (Lb90). Lb90 was added at three different levels to reach 5×10^3, 1×10^5, and 5×10^6 CFU/mL in the cheese milk and the cheeses so made were named as E1, E2, and E3, respectively. On each cheesemaking day, the milk was batch-pasteurized at 65 °C for 20 min (Briggiler-Marcó et al.,