J. Dairy Sci. 100:1–10
https://doi.org/10.3168/jds.2016-12418

Tracking Amazonian cheese microbial diversity: Development of an original, sustainable, and robust starter by freeze drying/spray drying

A. A. Ferreira,* S. Huang,†‡ Í.T. Perrone,* P. Schuck,‡§ G. Jan,‡§ and A. F. Carvalho*[1]
*Inovaleite Laboratory, Department of Food Technology, University Federal of Viçosa, 36570-000 Viçosa, Brazil
†Suzhou Key Laboratory of Green Chemical Engineering, School of Chemical and Environmental Engineering, College of Chemistry, Chemical Engineering and Material Science, Soochow University, 215123 Jiangsu, China
‡INRA, UMR1253 STLO, Science et Technologie du Lait et de l'OEuf, 35000 Rennes, France
§Agrocampus Ouest, UMR1253 STLO, 35000 Rennes, France

ABSTRACT

The Marajó cheese made with raw buffalo milk in the Amazon region of Brazil can be considered a good source of wild lactic acid bacteria strains with unexplored and promising characteristics. The aim of this study was to develop a potential probiotic starter culture for industrial applications using freeze drying and spray drying. A decrease in the survival rates of freeze-dried samples compared with spray-dried samples was noted. The spray-dried cultures remained approximately 10^9 cfu·g^{-1}, whereas the freeze-dried samples showed 10^7 cfu·g^{-1} after 60 d of storage at 4°C. All of the spray-dried samples showed a greater ability to decrease the pH in 10% skim milk over 24 h compared with the freeze-dried samples. The spray-dried samples showed a greater resistance to acidic conditions and to the presence of bile salts. In addition, under heat stress conditions, reduction was under 2 log cycles in all samples. Although the survival rate was similar among the evaluated samples after drying, the technological performance for skim milk showed some differences. This study may direct further investigations into how to preserve lactic acid bacteria probiotics to produce spray-dried starters that have a high number of viable cells which can then be used for industrial applications in a cost-effective way.

Key words: *Lactobacillus plantarum*, probiotic, sweet whey, Amazonian artisanal cheese

INTRODUCTION

In the food industry, starter/adjunct cultures are obtained by 2 methods of concentration/preservation: cultures obtained in frozen conditions and cultures obtained in a dried form, such as freeze drying. The major disadvantage to using frozen cultures is the cost of transport, storage, and manipulation (Carvalho et al., 2004b; Santivarangkna et al., 2007). Dried preparations have the added advantage of long-term preservation and handling and storage convenience. Freeze drying is a widespread technique; a large number of freeze-dried cultures are commercially available (Santivarangkna et al., 2007), These are widely used in the implementation of lactic acid bacteria (**LAB**) cultures (To and Etzel, 1997; Madhu et al., 2011; Kandil and El Soda, 2015).

Nevertheless, the freeze-drying process is expensive and complex, and can take days to complete for large product loads due to the slow energy and water transfer needed to dry the material. This, as well as the growing commercial interest in microbial culture starters, explains continuing research to develop alternative drying techniques (Carvalho et al., 2004b; Morgan et al., 2006; Santivarangkna et al., 2007; Silva et al., 2011).

Spray drying is a good example of a commonly used technique in industrial food drying today, which can also be used for drying bacteria. The energetic cost of spray drying is approximately 10 times less than that of freeze drying. The process' versatility and the considerable progress made through technical innovation have led to greater flexibility in meeting biotechnological requirements, especially low-heat treatments that help avoid loss of activity (Schuck et al., 2013).

Maximizing the survival of LAB cultures during drying and ensuring subsequent storage conditions for long periods is essential to maintaining technological characteristics and economic viability. The biological activity of a lactic culture that includes cell viability and its physiological state is necessary to evaluate the quality of a starter culture (Peighambardoust et al., 2011). Microbial cell survival throughout drying and storage depends on many factors, including initial concentration of microorganisms, growth conditions, growth medium, drying medium, storage conditions, and rehydration conditions (Carvalho et al., 2003, 2004b).

Received December 7, 2016.
Accepted May 27, 2017.
[1]Corresponding author: antoniofernandes@ufv.br

Even though freeze drying is the most common method used today, spray drying can also be used to dry probiotic cultures (Maciel et al., 2014; Soukoulis et al., 2014; Shokri et al., 2015; Utami et al., 2016). Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). However, these health benefits may depend on the viability of the probiotic cells and the maintenance of their probiotic properties in commercial cultures and food products during the storage (Rathnayaka, 2013).

The composition of the growth and drying medium can influence the protection during storage of dried cells (Carvalho et al., 2003, 2004a). Skim milk powder has been selected as the drying medium for most LAB cultures. Skim milk helps avoid cellular injury by stabilizing the cell membrane constituents (Carvalho et al., 2004; Zamora et al., 2006; Maciel et al., 2014) and it is one of the media used for bacterial protection during drying (Fu and Chen, 2011).

Whey is a food byproduct of cheese manufacturing. The large amount of lactose and whey proteins in sweet whey make it an ideal medium for growing LAB (Huang et al., 2016). Moreover, several studies have demonstrated that milk components could have protective effects on probiotics in adverse stress conditions (Ananta et al., 2005; Huang and Chen, 2013; Huang et al., 2014, 2016; Maciel et al., 2014).

Therefore, the use of a nutritional and low-cost drying medium that maintains the viability and functional properties of LAB cultures during storage, in association with a low-cost drying technology, can promote the development of an optimal probiotic/starter culture for industrial application (Maciel et al., 2014).

The use of wild strains is promising for starter/adjunct culture production because they contain characteristics that are particularly desirable for the food industry. The biodiversity found in artisanal cheeses allows for the identification of microbial strains with a wide range of technological and probiotic characteristics (Randazzo et al., 2007, 2009; Van Hoorde et al., 2008; Terzic-Vidojevic et al., 2014; de Freitas et al., 2015; Castro et al., 2016).

Brazil stands out in the production of artisanal buffalo milk cheese on the island of Marajó, in the state of Pará, located in the Amazon region. This cheese is known as Marajó cheese. The curd coagulation of Marajó is caused only by the autochthonous microbiota in raw milk and in the processing environment, which makes it a characteristic product of the region. Consequently, this cheese can be considered as a good source for isolating of the wild LAB strains for industrial application.

The aim of this study was to develop a potential probiotic starter culture for industrial applications using freeze drying and spray drying.

MATERIALS AND METHODS

Bacterial Strain and Growth Conditions

The strain *Lactobacillus plantarum* (UFV-Lb26) isolated from Marajó cheese and present in the culture collection of the INOVALEITE Laboratory, Department of Food Technology of the University Federal of Viçosa, Brazil, was selected to study the effects of freeze drying and spray drying. This strain exhibits the ability to produce diacetyl, as well as proteolitic activity, an acidifying and antimicrobial activity that attacks foodborne pathogens, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 7644 (data not shown), in addition to providing potential probiotic qualities such as low pH tolerance and high bile salt concentration of up to 1%. The strain was preserved at −20°C in Eppendorf tubes containing deMan, Rogosa, and Sharpe broth (**MRS**, Difco, Bordeaux, France) with 30% (vol/vol) glycerol added.

Preparation of Cells for Drying Experiments

The frozen culture (−20°C) was grown in 10 mL of sterilized *Lactobacilli* MRS broth at 30°C for 18 h (stationary phase). A 1% aliquot of the grown culture was transferred and regrown under the same conditions. Approximately 10^9 cfu·mL^{-1} cell density was obtained after incubation.

The cultures were transferred under aseptic conditions into 1-mL sterile Eppendorf tubes for the freeze-drying process, and into 1-L sterile centrifuge tubes for the spray-drying process. Next, the cultures were centrifuged at 8,000 × *g* for 5 min at 4°C. The supernatant was discarded and the harvested cells, in the form of pellets, were washed once using PBS at pH 7.0 (PBS, Difco, France). The cell suspensions were centrifuged again as previously. After discarding the supernatant, the pellets were suspended separately into the drying carrier medium with enhanced high DM, that is, 20% (wt/vol) of reconstituted skim milk powder (**RSM**; Lactalis, Bourgbarré, France), reconstituted sweet whey powder (**RSW**; Lactalis), and reconstituted sweet whey permeate powder (**RSWP**; Lactalis). The RSM contains all the components of milk in this composition, except for fat: whey proteins, lactose, vitamins, and minerals. While RSWP has no significant amount of protein, it does contain lactose, vitamins, and minerals.