Songklanakarin J. Sci. Technol.
34 (3), 241-245, May - Jun. 2012

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Survival rates of human-derived probiotic *Lactobacillus paracasei* SD1 in milk powder using spray drying

Rawee Teanpaisan[1,2]*, Aksorntong Chooruk[1,2], Akom Wannun[1,2], Santad Wichienchot[3], and Supatcharin Piwat[1,4]

[1] *Common Oral Diseases and Epidemiology Research Center,*

[2] *Department of Stomatology, Faculty of Dentistry,*

[3] *Nutraceutical and Functional Food Research and Development Center, Faculty of Agro-Industry,*

[4] *Department of Preventive Dentistry,*
Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla, 90110 Thailand.

Received 18 December 2011; Accepted 24 April 2012

Abstract

Spray drying of skim milk has been evaluated as a mean of preserving *Lactobacillus paracasei* SD1, which is a human-derived strain with probiotic potential. Our experiments revealed that an air outlet temperature of 80°C was optimal for spray drying. Such condition resulted in powder with moisture contents of 3.44±0.85% and viable counts of $7.5\pm0.20 \times 10^8$ CFU/g. Although the probiotic strain appeared to be stressed from spray drying, bacteriocin production by *L. paracasei* SD1 was not affected by the process. The level of survival of *L. paracasei* SD1 remained constant at 10^8 CFU/g during 6 months of powder storage at 4°C, while a decline in the level of survival was observed for storage of powder at 25°C. Our data demonstrate that spray drying may be a cost-effective way to produce a large quantity of the probiotic *L. paracasei* SD1.

Keywords: probiotic, *Lactobacillus paracasei* SD1, skim milk, spray drying

1. Introduction

Probiotic microorganisms play an important role in promoting and maintaining health (Salminen *et al.*, 1998; Parvez *et al*., 2006), and they have stimulated considerable interest in incorporating these into functional foods and pharmaceutical products. *Lactobacillus* has recently been proposed to promote oral health. Most studies have reported an inhibitory activity of oral *Lactobacillus* against cariogenic *Streptococcus* (Simark-Mattsson *et al.,* 2007; Teanpaisan *et al.,* 2011), and some also have demonstrated growth suppression of periodontal pathogens (Koll-Klais *et al.,* 2005; Teanpaisan *et al.,* 2011). Thus, certain strains of *Lactobacillus* have been suggested to be used as probiotics for oral health. By definition, probiotics are "living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Guarner and Schaafsma, 1998), and it is recommended that probiotic products contain at least 10^7 live microorganisms per g or per ml (Ishibashi and Shimamura, 1993). Therefore, from a commercial point of view, an inexpensive method for large-scale production of cultures containing high levels of viable probiotic cells in a form suitable for product applications is highly desirable.

Spray drying is one of the predominant processing tools used in the dairy industry. It can be used to produce large amounts of dairy ingredients relatively inexpensively and it has been estimated that the cost of spray drying is six

* Corresponding author.
Email address: rawee.t@psu.ac.th

times lower per kilogram of water removed than the cost of freeze-drying (Knorr, 1998). Spray-dried powders can be transported at a low cost and can be stored in a stable form for prolonged periods. However, there are obvious challenges associated with using spray drying to produce viable cultures, including the requirement that the microorganisms survive the relatively high temperatures used (Daemen *et al*., 1982). Previous studies have investigated the use of spray drying as a way to preserve yogurt with viable microorganisms (Kim and Bhowmik, 1990), and dairy starter cultures, such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Teixeira *et al*., 1995a). In addition to maintaining the viability of probiotic cultures, it is important that probiotic properties are maintained following the spray-drying process. Although spray dried probiotic cultures are available commercially, the previously published data related to spray drying of such microorganisms is limited. Some studies have been undertaken to investigate the survival during spray drying of *Lactobacillus acidophilus* cultures chosen for their health-promoting properties (Prajapati *et al*., 1987).

Due to the advantages of spray drying, in the present study we investigated the use of this method as a way to preserve human-derived *Lactobacillus paracasei* SD1. The bacterium which we used has previously been well characterized with respect to its probiotic properties of antimicrobial effect against oral pathogens (Teanpaisan *et al.,* 2011). The aim of the present study was to investigate spray drying as a method for pilot-scale production of dairy-based powder containing this probiotic *Lactobacillus* culture.

2. Material and Methods

2.1 Bacterial strains and culture conditions

Probiotic strain *L. paracasei* SD1 was previously isolated from the human oral cavity, and was identified as *L. paracasei* according to 16S-rRNA gene profiles by restriction fragment length polymorphism analysis (PCR-RFLP) and protein profiles by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Teanpaisan and Dahlén, 2006). The strain was stored at -80°C until used.

2.2 Spray drying of milk powder containing probiotic

For spray-drying purpose, the *L. paracasei* SD1 strain was cultured as follows. The strain was recovered from -80ºC on a MRS agar plate and inoculated into a 50 ml MRS broth overnight in anaerobic condition (80% N_2, 10% H_2, and 10% CO_2) at 37ºC. The culture was then added into a 450 ml MRS broth and the culture was continued in the same condition for 48 h. Cells were harvested by centrifugation (1,500 g for 5 min) from MRS broth, and washed 3 times with 0.85% NaCl before used.

The concentrated culture of *L. paracasei* SD1 was inoculated to 1% final concentration in 3 liters of heat-treated (50°C, 30 min) 20% reconstituted skim milk which gave an initial number of *L. paracasei* SD1 of approximately 10^9 CFU/ml. The mixture was then spray dried with a laboratory scale spray dryer (model B191 Buchi mini spray dryer; Flawil, Switzerland) using a constant inlet air temperature of 170°C. The mixture was atomized and sprayed into the drying chamber, and the product dried almost instantaneously. To investigate the effect of the outlet air temperature, the feed rate was varied to obtain outlet temperatures ranging from 60°C to 90°C. Each trial was conducted in triplicate. The powders of each trial was then stored in sealed polyethylene bags at 4°C and 25°C, and probiotic viability was assessed over time.

2.3 Determination of probiotic viability in spray-dried milk powder

Viability of the probiotic *L. paracasei* SD1 in the inoculated milk preparations was assessed before spray drying and in the resulting powders by examining triplicate MRS pour plates after 3 days of anaerobic incubation at 37°C. To 0.1 g of powder, 9.9 ml of diluent was added (1:100 dilution); the preparation was allowed to rehydrate for 1 h and then diluted further with diluent, and appropriate dilutions were pour plated. The survival percentage at each of the outlet temperatures tested was calculated as follows:

$$\% \text{ survival} = (N/N_0) \times 100,$$

where N_0 is the number of bacteria per gram of dry ma tter before drying and N is the number of bacteria per gram of dry matter in the powder.

2.4 Determination of moisture contents of spray-dried powders

The moisture contents of spray-dried skim milk powders were determined in triplicate by oven drying the powders at 102°C, determining the difference in weight, and expressing the weight loss as a percentage of the powder weight (International Dairy Federation, 1993). A moisture content of 4% is recommended for skim milk powder (Masters, 1985).

2.5 Bacteriocin assay of *L. paracasei* SD1

Before and after spray drying, the inhibitory effect of *L. paracasei* SD1 against *Streptococcus mutans* was assessed by an agar overlay method (Teanpaisan *et al.,* 2011). In brief, *L. paracasei* SD1 (producer strains) was inoculated on the surface of the brain heart infusion agar and incubated anaerobically (80% N_2, 10% H_2, and 10% CO_2) for 24-48 h at 37ºC to develop visible macro-colonies. *Streptococcus mutans* ATCC 25175 was used as an indicator strain. The indicator strain was precultivated in the brain heart infusion broth (BHI) and the suspension of cells was adjusted to an

optical density (OD) 0.25 at 600 nm. Thereafter, 5 ml of BHI soft agar (7 g/l agar) was seeded with 100 ul of an overnight culture of the indicator strain and immediately poured over the macro-colonies of the producer. The plate was incubated anaerobically at 37°C for 24 h to generate an inhibitory zone.

3. Results and Discussion

In the present study, we investigated the use of spray drying as a way to prepare dairy-based powder harboring high numbers of viable cells of the oral human-derived strain *L. paracasei* SD1.

The initial spray drying experiments were performed to determine the optimum outlet temperature for probiotic viability which yielded powders with moisture contents that were not greater than 4% (Masters, 1985). The data obtained in these trials indicated that the optimal drying conditions for *L. paracasei* SD1 were an air inlet temperature of 170°C and an air outlet temperature of 80 and 90°C, which yielded powders with moisture contents within the recommended range. The moisture contents of the powders increased as the outlet temperature decreased ranging from 2.36±0.18 to 6.89±0.04% at 90°C to 60°C (Figure 1), which were similar to previous reports during preparation of spray dried powders containing other microorganisms (Espina and Packard, 1979; Johnson and Etzel 1993; Gardiner *et al*., 2000). In general, an air outlet temperature of 80°C to 90°C was necessary in order to obtain powders with moisture contents that did not exceed the level required.

The probiotic survival rate decreased during spray drying as the outlet temperature increased; this is an expected result of the present study which confirms previous findings where the outlet temperature was found to have a large effect on culture viability during spray drying (Espina and Packard, 1979; Johnson and Etzel, 1993; Kim and Bhowmik, 1990; Gardiner *et al*., 2000). The recovery number of *L. paracasei* SD1 during spray drying was 2.5×10^9 CFU/ml, 9.5×10^8 CFU/ml, 7.5×10^8 CFU/ml and 8.0×10^7 CFU/ml at an outlet temperature of 60°C, 70°C, 80°C and 90°C, respectively. The survival rates of *L. paracasei* SD1 during spray drying ranged over 46%, 33%, 19% and 4% at an outlet temperatures of 60°C, 70°C, 80°C and 90°C, respectively (Figure 1). The survival rate was close to the survival rate of *Lactobacillus helviticus,* which was 15% at an outlet temperature of 82°C (Johnson and Etzel 1993). However, the survival rates obtained in this study were higher than those previously reported for *Lactobacillus acidophilus* and *Lactobacillus salivarius*, which were 8.2% and 11% at an outlet temperatures of 75°C and 60°C, respectively (Espina and Packard 1979; Gardiner *et al*., 2000). These data highlight the importance of optimization of process parameters, especially, inlet and outlet temperatures.

To explore probiotic survival in spray-dried powders during storage, powder was produced by spray drying at air outlet temperatures ranging from 60°C to 90°C. The powders of each trial was then stored in sealed polyethylene bags at 4°C and 25°C, and probiotic viability was assessed over a 6-month period. Following 6 months of storage, the maximum survival rate of *L. paracasei* SD1 in the skim milk powders (99%) occurred at 4°C (Figure 2). The survival rates of the strain decreased more rapidly during storage at 25°C, and the survival rates were close to 0% after 6 months of storage (Figure 2). Previous studies have also shown that temperature is critical for microbial survival during storage, and higher survival rates have been obtained at lower storage temperatures (Johnson and Etzel 1993; Teixeira *et al.*, 1995b; Gardiner *et al*., 2000).

During storage, the moisture contents of the spray dried powders produced at different outlet temperatures gradually increased, particularly when the powders were stored at 25°C (Figure 3). In general, an air outlet temperature of 80°C and 90°C was necessary in order to obtain

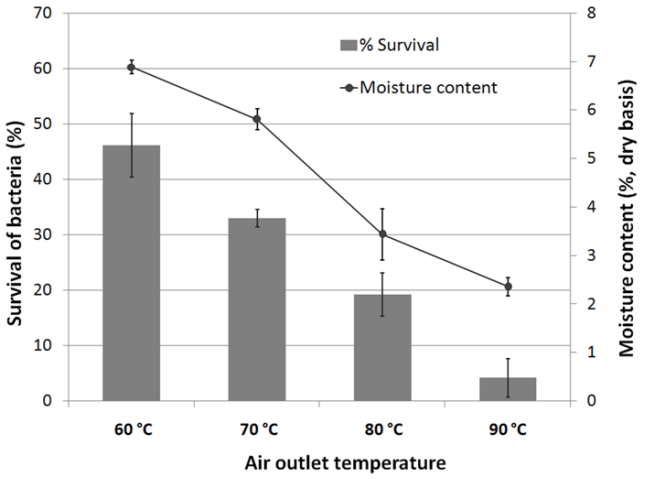

Figure 1. Survival rate (%) of *L. paracasei* SD1 and moisture content (%) of spray-dried skim milk powders at various air outlet temperatures.

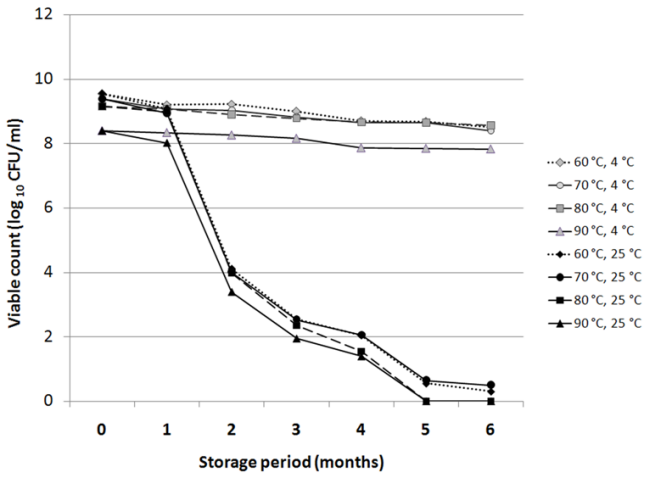

Figure 2. Survival of *L. paracasei* SD1 in spray-dried skim milk powders during storage at 4°C (grey lines) and 25°C (black lines).

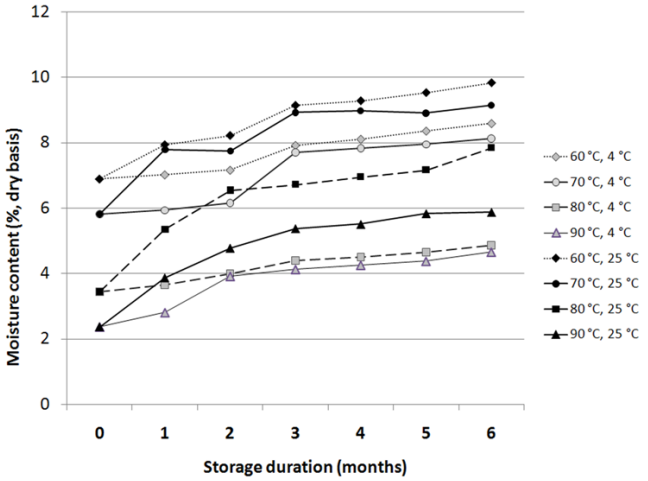

Figure 3. Moisture content of spray-dried skim milk powders during storage at 4°C (grey lines) and 25°C (black lines). Refrigerator (4°C), relative humidity approximately 30-40 %; Room air (25°C), relative humidity approximately 70-80 %

powders with moisture contents that did not exceed the level required (4%) and to provide a sufficiently viable count for prolonged powder storage life. Such results as in this study suggest that refrigerated storage is necessary for optimal culture viability in spray-dried powders over time.

To explore the effect of spray drying on bacteriocin production, the bacteriocin ability of strains before and after spray drying were compared. It has been previously shown that *L. paracasei* SD1 produces a broad-spectrum bacteriocin that exhibits activity against oral pathogens such as *Streptococcus mutans, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* and *Tanerella forsythia* (Teanpaisan *et al*., 2011). Bacteriocin production is a desirable trait for probiotic cultures (Collins *et al*., 1998) and may be used to competitively exclude undesirable microorganisms in the oral cavity, thereby playing a role in probiotic persistence in the host. We investigated the effect of spray drying on the ability of *L. paracasei* SD1 to produce bacteriocin. Following isolation from spray-dried powders produced at a range of outlet temperatures, the *L. paracasei* SD1 strain retained its ability to produce bacteriocin, showing that it could do so even at outlet temperatures as high as 90°C. It was shown that the potential probiotic trait related to bacteriocin production was not affected; an example was shown as Figure 4.

It has been well documented that both lactobacilli and lactococci retained their abilities to produce bacteriocin (Daemen *et al*., 1982). The presence of active bacteriocin ability of *L. paracasei* SD1 in powder after spray drying in this study means that in addition to providing the potential to add viable probiotic microorganism to food products, the powder may also play a role in inhibiting pathogenic microorganisms in the oral cavity via food systems. Although it was found that the spray-drying process did not affect bacteriocin production by *L. paracasei* SD1, survival of the spray-dried cultures under conditions that are present in the oral cavity also need be investigated.

4. Conclusions

In this study, it was shown that the *L. paracasei* SD1 strain, which was selected on the basis of its probiotic properties, varied considerably in the ability to survive during the spray-drying process. Our findings highlight the need to take into consideration the technological properties of probiotic strains and emphasize the importance of strain selection with regard to processing, as well as health-promoting properties. In this respect, it was found that probiotic *L. paracasei* SD1 remained viable during spray drying and determined an optimal outlet temperature for cell viability and moisture content of the powders. In addition, the spray-drying process did not affect bacteriocin production. In conclusion, spray drying is potentially a useful process for large-scale production of human probiotic *Lactobacillus* strains in a form suitable for transport and storage. Furthermore, given the numerous applications of skim milk powders, not only in dairy products but also in foods such as instant desserts and confectionery products, it is possible that the resulting culture-containing powders could be used in a wide range of functional food applications.

Acknowledgement

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, and the National Science and Technology Development Agency (NSTDA) of Thailand.

References

Collins, J. K., Thornton, G. and O'Sullivan, G. 1998. Selection of probiotic strains for human applications. International Dairy Journal. 8, 487-490.

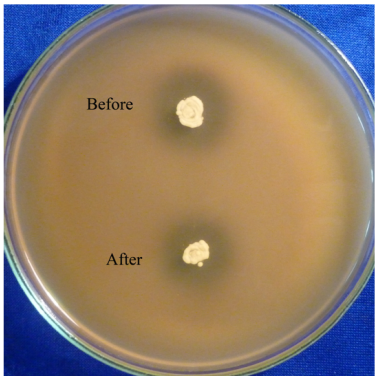

Figure 4. Bacteriocin assay of *L. paracasei* SD1, strain tested before and after spray drying.

Daemen, A. L. H. and van der Stege, H. J. 1982. The destruction of enzymes and bacteria during the spray drying of milk and whey. 2. The effect of the drying conditions. Netherlands Milk and Dairy Journal. 36, 211-229.

Espina, F. and Packard, V. S. 1979. Survival of *Lactobacillus acidophilus* in a spray-drying process. Journal of Food Protection. 42, 149-152.

Gardiner, G.E., O'Sullivan, E, Kelly, J., Auty, M.A., Fitzgerald, G.F., Collins, J.K., Ross, R.P. and Stanton, C. 2000. Comparative survival rates of human-derived pro-biotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Applied and Environmental Microbiololy. 66, 2605-12.

Guarner, F. and Schaafsma, G. J. 1998. Probiotics. International Journal of Food Microbiology. 39, 237-238.

International Dairy Federation. 1993. Dried milk and dried cream. Determination of water content. International Dairy Federation standard 26A. International Dairy Federation, Brussels, Belgium.

Ishibashi, N. and Shimamura S. 1993. Bifidobacteria: research and development in Japan. Food Technology. 46, 126-135.

Johnson, J. A. C. and Etzel, M. R. 1993. Inactivation of lactic acid bacteria during spray drying, p. 98–107. *In* G. V. Barbosa-Canovas and M. R. Okos (ed.), Food dehydration. Institute of Chemical Engineering, New York, N.Y.

Kim, S. S. and Bhowmik, S. R. 1990. Survival of lactic acid bacteria during spray drying of plain yogurt. Journal of Food Science. 55, 1008–1010.

Knorr, D. 1998. Technology aspects related to microorganisms in functional foods. Trends in Food Science & Technology. 9, 295-306.

Koll-Klais, P., Mandar, R., Leibur, E., Marcotte, H., Hammarstrom, L. and Mikelsaar, M. 2005. Oral lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity. Oral Microbiology and Immunology. 20, 354-361.

Masters, K. 1985. Analytical methods and properties of dried dairy products, p. 393-403. *In* R. Hansen (ed.), Evaporation, membrane filtration and spray drying in milk powder and cheese production. North European Dairy Journal, Vanlose, Denmark.

Parvez, S., Malik, K.A., Ah Kang, S. and Kim, H.Y. 2006. Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology. 100, 1171-1185.

Prajapati, J. B., Shah, R. K. and Dave, J. M. 1987. Survival of *Lactobacillus acidophilus* in blended-spray dried acidophilus preparations. Australian Journal of Dairy Technology. 42, 17–21.

Salminen, S., Ouwehand, A. C. and Isolauri, E. 1998. Clinical applications of probiotic bacteria. International Dairy Journal. 8, 563-572.

Simark-Mattsson, C., Emilson, C.G., Hakansson, E.G., Jacobsson, C., Roos, K. and Holm, S. 2007. *Lactobacillus*-mediated interference of mutans streptococci in caries-free vs. caries-active subjects. European Journal of Oral Science. 115, 308-314.

Teanpaisan, R., Piwat, S. and Dahlén, G. 2011. Inhibitory effect of oral *Lactobacillus* against oral pathogens. Letters in Applied Microbiology. 53, 452-459.

Teanpaisan, R. and Dahlén, G. 2006. Use of polymerase chain reaction techniques and sodium dodecyl sulfate-polyacrylamide gel electrophoresis for differentiation of oral *Lactobacillus* species. Oral Microbiology and Immunology. 21, 79-83.

Teixeira, P., Castro, H. and Kirby, R. 1995a. Spray drying as a method for preparing concentrated cultures of *Lactobacillus bulgaricus*. Journal of Applied Bacteriology. 78, 456-462.

Teixeira, P. C., Castro, M. H., Malcata, F. X and Kirby, R. M. 1995b. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* following spray drying. Journal of Dairy Science. 78, 1025-1031.