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Survival, fermentation activity and storage stability of spray dried *Lactococcus lactis* produced via different atomization regimes

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ABSTRACT

Dried powders containing *Lactococcus lactis* ssp. *cremoris* were produced using laboratory and pilot scale spray dryers with lactose:whey protein isolate (3:1) as a protective medium. The effects of storage temperature (25, 4 and −18 °C) and time (30, 60 and 90 days) were studied. The survival and fermentation activity of the dried bacterial cells were significantly lower when the powders were stored at 25 °C compared to those stored at 4 and −18 °C; powders stored at 4 and −18 °C were statistically similar. The survival and fermentation activity of bacterial cells obtained from a laboratory scale two-fluid nozzle spray dryer were found to be higher than those of cells obtained from a pilot scale two-fluid spray dryer. A rotary wheel atomizer gave significantly higher survival and activity in the same dryer. These observations are consistent with cell damage due to high characteristic shear rates in the atomization process in nozzle type atomizers. The presence of ascorbic acid (oxygen scavenger) in the powder composition was found to improve both the survival and the maintenance of fermentation activity of the dried bacterial cells significantly during storage. The survival and fermentation activity of dried bacterial cells in stored powders indicated that these parameters are system-specific and can be strongly affected by the storage temperature and presence or absence of antioxidant, and also by upstream processing conditions such as the mode of atomization and presence or absence of antioxidants in the dryer feed.

1. Introduction

Drying of bacterial cultures for use in dairy fermentations and in other food and therapeutic applications is common commercial practice. There is continuing research interest in improving the application of drying techniques to various food-related bacteria, including *Lactobacillus paracasei* (Gardiner et al., 2000), *Lactobacillus curvatus* (Mauriello et al., 1999), *Lactobacillus rhamnosus* (Corcoran et al., 2005), *Brevibacterium linens* (To and Etzel, 1997b), *Lactobacillus helveticus* (Johnson and Etzel, 1995), and *Streptococcus thermophilus* and *Lactobacillus debrueckii* ssp. *bulgaricus* (Kim and Bhowmik, 1990). Investigations to improve their viability have examined various aspects such as intrinsic features of bacterial cultures (Bozoglu et al., 1987; Palmfeldt and Hahn-Hagerdal, 2000), the effect of growth media and growth conditions (Champagne and Gardner, 2001; Linders et al., 1997), cell harvesting conditions (Champagne and Gardner, 2002; van de Guchte et al., 2002), stress adaptation (Broadbent and Lin, 1999; Prasad et al., 2003), application of protective agents (Koster et al., 2000; Leslie et al., 1994; Oldenhof et al., 2005), manipulating the rehydration conditions (Abadias et al., 2001; Zhao and Zhang, 2005) and improving the packaging and storage conditions (Brennan et al., 1983; Buitink et al., 2000; Castro et al., 1995).

Spray drying has advantages over other drying methods such as cost-effectiveness, relative ease in operation, easy scale-up for large throughputs and ready availability of suitable equipment at various scales (Gibbs et al., 1999; Horaczek and Viernstein, 2004). However, bacterial survival is generally poor. Storage conditions are considered to be one of the key factors which directly affect the cell viability and fermentation activity of spray-dried bacterial starter cultures (Peighambardoust et al., 2011). Several factors during storage affect survival and fermentation activity, including reaction with oxygen, moisture, light, microbial contamination and elevated temperature (Morgan et al., 2006). Survival decreases during storage, and survival is generally reported to be higher when lower storage temperatures are used (Boza et al., 2004; Corcoran et al., 2004; Desmond et al., 2002; Silva et al., 2002). Teixeria et al. (1996) suggested that oxidation of the fatty acids of membrane lipids is the most likely cause of death of microbial cells during storage. According to these authors, the lipid composition of the bacterial cell membrane changes due to the increased lipid oxidation during storage. Better viability of bacteria (*Lactobacillus bulgaricus* and *S. thermophilus*) was reported when the spray dried bacterial cultures were stored under vacuum and in nitrogen-rich conditions compared to those stored in normal atmospheric air (Bozoglu et al., 1987).

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However, there are no reported studies comparing the survival and fermentation activity of bacterial cells obtained from laboratory- and pilot-scale spray drying operations as a function of storage temperature and storage time, and relatively few studies of the common cheese starter bacterium *Lactococcus lactis*. This study was aimed at investigating these issues as they affect preservation and use of a *L. lactis* ssp. *cremoris* dairy starter culture.

2. Material and methods

2.1. Materials

L. lactis ssp. *cremoris* strain ASCC930119 (Pillidge et al., 2009) from the Dairy Innovation Australia culture collection was cultured overnight in 15 ml of M17 broth (Oxoid, Australia) at 30 °C under static conditions. The resulting culture was transferred to 1 L of M17 broth at 30 °C under the same conditions. Cells were harvested by centrifugation at 2500xg for 5 min and then suspended in lactose:whey protein isolate (3:1) solution using gentle agitation at 25 °C and adjusted to *ca.*10^{10} cells/ml.

α-Lactose monohydrate (99.8% purity, Sigma–Aldrich, Australia), whey protein isolate (WPI, protein content 94.5%, obtained by courtesy of Murray Goulburn Cooperative, Australia) and L-ascorbic acid (Chem-Supply, Australia) were used as received.

2.2. Methods

2.2.1. Solution preparation

As described by Ghandi et al. (2012b), lactose:WPI (3:1; total solids 35% w/w) in deionized water was prepared by heating at 45 ± 1 °C and gently agitating with a magnetic stirrer. A 10% (w/w) ascorbic acid solution was prepared, and added to the bacterial suspension (in lactose:WPI, pH = 6.5 ± 0.1) just before spray drying, to a concentration of 0.7% (w/w) and pH 5.5.

2.2.2. Powder production

Laboratory-scale spray drying was carried out using a Buchi 290 dryer (Buchi, Switzerland) with water evaporation capacity of 1 L/h. The bacterial suspension was pneumatically atomized using a two-fluid nozzle (nozzle diameter, inner D_i = 1.2 mm; outer D_o = 1.4 mm). The flow rate of the drying air was maintained at 35 m^3/h while the aspiration was at 100%.

Pilot-scale drying used a Drytec™ Spray Dryer (Drytec, Tonbridge United Kingdom; water evaporation capacity: 8 L/h; exhaust fan capacity: 160 m^3/h) fitted with either a rotary wheel atomizer or a two-fluid nozzle (inner D_i = 1.6 mm; outer D_o = 2.4 - mm; atomizing air pressure: 550 kPa) as was used in previous studies (Crittenden et al., 2006; Ying et al., 2012).

Previous work had determined that inlet and outlet air temperatures of 130 and 65 °C, respectively, gave acceptable bacterial survival and residual moisture content not exceeding 5% (w/w) (Ghandi et al., 2012b). Powder was collected in a product container connected to the bottom of the cyclone separator and cooled using an electric fan.

2.2.3. Estimation of shear rates

The characteristic shear rates in the atomization process in two-fluid nozzle both in laboratory and pilot scale spray dryers was calculated using Hede et al.'s method (Hede et al., 2008). Assuming momentum is transferred between the liquid feed and atomizing gas in the mixing zone and both leave the atomization zone at constant average velocity (v_{av}), the shear rate at the exit of atomizing nozzle was calculated using Eq. (1), below.

$$\dot{\gamma} = \frac{2(v_{av} - v_L)}{D_L} \quad (1)$$

where ($\dot{\gamma}$) is the characteristic shear rate (s^{-1}) and D_L is the inner nozzle diameter (m). v_{av} is the exit average velocity of both the atomizing gas and liquid feed ($m\,s^{-1}$). v_L is the mean velocity of the liquid feed within the nozzle before exit ($m\,s^{-1}$). The mean velocity of atomizing gas within the nozzle before exit is embedded in the calculation of v_{av}.

The characteristic shear rate in a rotary wheel atomizer was calculated as suggested by Garcia et al., represented by Eq. (2) (Garcia et al., 1997).

$$\dot{\gamma} = 0.8\,R\sqrt{\frac{\rho(2\pi N)^3}{\mu}} \quad (2)$$

where R is the radial distance from the centre to the edge of the disc (in this case: 0.02675 m); ρ and μ are the density ($kg\,m^{-3}$) and viscosity (Pa·s) of the liquid feed, and N is the rotational velocity of the disc (20,000 rpm, expressed as 333.33 revolution s^{-1}). The detailed procedure for calculating these shear rates is presented in our earlier paper (Ghandi et al., 2012b).

2.2.4. Storage

The bacterial powders were stored for 90 days at three different temperatures (25, 4 and −18 °C) in dark glass vials under nitrogen flush, with each vial vacuum-sealed in a separate plastic bag. The storage duration was chosen based on the typical duration used in shelf life studies (Fonseca et al., 2000; To and Etzel, 1997b) and in many industrial settings. Bacterial survival was measured at intervals of 15 days across the 90 days storage trial. Acid production activity was evaluated at intervals of 30 days.

2.2.5. Bacterial survival during storage

Spray dried powder (0.1 g) was rehydrated in 9.9 mL sterile peptone water (1% w/v, pH = 7.0 ± 0.1) (Gardiner et al., 2000). Live and dead cells were counted by a fluorescein diacetate (FDA) staining method (Lentini, 1993; Ghandi et al., 2012a). The initial viable cell concentration of the fresh culture was determined at the time of drying. Survival of bacteria was expressed as a percentage of the live bacterial cells immediately after spray drying (before the powders were stored = zero storage time).

2.2.6. Measurement of fermentation activity

Fermentation activity is described as the ability of the bacteria to produce lactic acid, and reflects the number of live cells and their health. Dried culture (0.7 g of spray dried powder) was rehydrated in 35 mL sterile non-fat skim milk (10%, w/w) and stirred in a water-bath at 30 °C for 2 min before starting the fermentation tests. These fermentation tests were carried out for 24 h using a water bath maintained at 30 °C. The change in pH of the suspension was taken as the measure of fermentation activity. The pH changes were monitored using a data logger (8-Channel Ion analyser, NICO 2000 Ltd., U.K.); pH *vs* time data were recorded. Rates of pH change (dpH/dt) throughout the fermentations were estimated using the formulae described by Ghandi et al. (2012a) for droplet mass loss analysis.

2.2.7. Statistical analysis

Statistical analysis was performed using ANOVA. The mean values and standard deviations were calculated from triplicate experimental data. Differences were compared by Tukey test for all experiments and were considered significant at $p < 0.05$. All statistical analyses were performed with Minitab 15 software (Minitab, Australia).