Enhanced viability of *Bacillus coagulans* after spray drying with calcium lactate, storage and re-hydration

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The present work has provided an alternative probiotic preparation, which is easily scalable for commercial operations in eco-friendly and cost-effective manner. Spray drying being a proven technology compared to convective current, infra-red exposure and lyophilization, it will make visible impact on production, sale and use of *Bacillus coagulans* as an effective probiotic preparation.

**Keywords:** Probiotics, *Bacillus coagulans*, Calcium lactate as protectant, Spray drying

Use of freeze-drying (lyophilization) technology for commercial production of probiotics is cost-prohibitive due to low yield as a result of three phases of drying system, namely freezing, primary drying and secondary drying. Secondly, there are practical limitations in using this technology on commercial scale. Thirdly, the viability of cells is doubtful if the cold chain is broken. In contrast, spray drying provides an economical technology due to higher productivity per unit time and practicability ease on large scale. A number of studies have reported survival rate and performance of a variety of probiotic cultures after spray drying as a function of outlet temperature and drying medium. During spray drying, removal of bacterial cells-bound water leads to damage of surface proteins, cell membrane and cell wall, thereby destabilizing secondary structure and functional integrity of RNA and DNA by tampering different types of weak bonding. Towards minimizing damage of these cellular vital components, a variety of protectants like skimmed milk powder, whey proteins, dextran, polyethylene glycol (PEG), sucrose, lactose, glucose, trehalose, glycerol, sorbitol, adonitol, betaine etc have been studied into the drying medium during spray drying to protect their viability. The use of gum acacia in the spray drying medium provided enhanced probiotic survival of *L. paracasei* NFBC 338, which displayed 10-fold greater survival than control cells with reconstituted 20% skimmed milk (RSM) at air outlet temperature of 100-105°C. Keeping the above protectants in mind, application of maltodextrin, calcium gluconate, calcium lactate and *Spirulina* as protectants has been explored to see their protective effect on the survival of *Bacillus coagulans* (earlier known as *Lactobacillus sporogens*). Efforts made in this direction constitute the theme of the present communication.

**Experimental Procedure**

**Materials**

Calcium lactate was procured from KCP Sugar and Industrial Corporation Ltd., Vuyyuru (AP); calcium gluconate from Shan Par Pvt. Ltd., Mumbai; Maltodextrin, liquid glucose and corn steep liquor (CSL) from Bharat Starch Industries Ltd., Yamuna Nagar; *Spirulina* from HDV Chem-Pharma Pvt. Ltd., Chennai and analytical reagents from S.d. Fine Chemicals, Mumbai.

**Inoculum preparation**

*Bacillus coagulans*, isolated from Sporolac powder (Uni-Sankyo Ltd, Maharashtra, (B.No. A4SP009M3), was sub-cultured and preserved on MRS medium (Hi-Media, Mumbai).

**Experimental set up**

*Bacillus coagulans* was grown using pre-sterilized (121°C, 30 min) medium, per liter comprised of CSL
10 g, liquid glucose (60% DS) 5 g, peptone 5 g, magnesium sulphate 0.05 g and calcium chloride 0.05 g (pH 6.8). Upon cooling to 38°C, the medium was inoculated with *Bacillus coagulans* (24 h old, 5% *v/v*, 7x10⁶ cells/mL, pH 6.5) and fermentation carried out on an incubator shaker (Orbitech, 38 ±1°C, 200 rpm, 72 h).

After complete sporulation, one liter *Bacillus coagulans* broth was fortified individually with 200 g of pre-sterilized (i) maltodextrin, (ii) calcium gluconate, (iii) calcium lactate and (iv) *Spirulina* at 45±1°C for 30 min under aseptic conditions. The mixed broth was taken for spray drying using lab spray dryer (Yamato, Japan).

### Spray drying

Spray drying of *Bacillus coagulans* was accomplished under optimized conditions (air inlet temperature 180±1°C, air pressure 2.5 kg/cm², flow rate @ 25 mL/min and air outlet temperature 90±1°C).

### Analytical methods

During fermentation, (i) pH was monitored using digital pH-meter (Elico), (ii) microscopic examination of *Bacillus coagulans* for growth and spore formation was monitored by using phase contrast microscope (Magnus MLX-DX; Olympus, India) and (iii) spore count was performed using cell counter (Neubauer Chamber, Germany).

### Storage conditions

The spray dried preparations were stored in air-tight containers at 30±1°C, 55±5% RH for 12 months. During the storage period, viable count was monitored by serial dilution through spread technique at tri-monthly intervals.

### Results and Discussion

Probiotics are defined as live microorganisms, which upon administration in an adequate amount confer health benefit(s) to the consumers⁸. The microorganisms most frequently used as probiotic agents are *Lactobacilli*, *Bifidobacteria* and *Yeasts*. They are known since long time and increasingly gaining scientific interest as functional foods in modern life style, self-care and complementary medicine⁹,¹⁰. Therefore, they have been exploited commercially, only in the last two decades, using (i) different protectants, (ii) different microorganisms and (iii) for preventing/treating diseases by marketing of a broad range of products¹¹,¹².

In view of keeping the cells intact for their health-imparting effect(s) upon administration to human beings, freeze drying and spray drying methods have been prevalent during their commercial production⁶. In freeze drying method, in the first step of drying, cells are frozen and dried by sublimation under high vacuum using leak-proof vials¹. The freeze drying method provides higher % survival of cells as compared to that of spray drying method, due to lower temperature and mild shock (if any) to the microbial cells¹³.

On the contrary, spray drying method involves injection of cell mass fortified in spray drying medium and its exposure is controlled at (i) higher velocity of hot air, (ii) inlet and outlet temperature and (iii) under mild vacuum for a short duration⁶. Consequently, water removal can lead to de-stabilization of the structural integrity of cellular components, resulting in a loss or impairment of designated function(s). The primary target area of de-stabilization during drying is reported to be a lipid fraction of the cell membrane being affected due to peroxidation³,⁴. This may result in reduced efficacy of DNA replication, transcription and translation¹² and in turn, live character, as also health-imparting effect.

In order to achieve optimum results after spray drying of probiotics, therefore, attention must be focused on (i) the choice of stabilizer chemicals, (ii) their vital proprieties for cell survival and (iii) fine tuning of conditions of spray drying to minimize damage to the cellular components. In this regard, various researchers have noted that calcium in milk provides protective coating on bacterial cell wall and thereby increases their survival after dehydration¹⁵.

Towards this objective, under optimized conditions, calcium lactate, calcium gluconate, *Spirulina* and maltodextrin have been explored as probiotic protectants during spray drying. The results are summarized in Table 1.

Table 1: Impact of Storage Conditions on Survival of *Bacillus coagulans*<sup><small>16</small></sup>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Initial Survival</th>
<th>Final Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze Drying</td>
<td>6.3 x 10⁶</td>
<td>0.015 x 10⁶</td>
</tr>
<tr>
<td>Spray Drying</td>
<td>6.3 x 10⁶</td>
<td>0.24 x 10⁶</td>
</tr>
</tbody>
</table>

From Table 1, it is clear that under optimized conditions of spray drying, the viable count of the spores is variable as a function of protecting chemical used for drying. Initial viable count was 6.3-8.5 x 10⁶ cells in the spray dried preparations. The viable count was monitored at tri-monthly frequency as a function of storage period. Maltodextrin showed decreasing rate of survival of *Bacillus coagulans*, with the result viable count was reduced from 6.3 x 10⁶ to 0.015 x 10⁶ (merely 0.24% survival). Maltodextrin was purposefully explored in view of its water holding and
The probiotic preparation spray dried with calcium lactate has shown most likely reason to promote 73% spore survival. Any explicable reason, presumably appears to be the gain to the finished product, which in the absence of any property, calcium lactate has imparted more weight to hold about 23-30% water. Due to this property, calcium lactate has imparted more weight gain to the finished product, which in the absence of any explicable reason, presumably appears to be the most likely reason to promote 73% spore survival.

Table 1 — Efficacy of maltodextrin, calcium gluconate, Spirulina and calcium lactate for viability of Lactobacillus coagulans

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Protectant used for spray drying</th>
<th>Initial cell count/g (×10^8)</th>
<th>Product collected (g)</th>
<th>Moisture content (%)</th>
<th>Cell count/g after 3 months (×10^8)</th>
<th>Cell count/g after 6 months (×10^8)</th>
<th>Cell count/g after 9 months (×10^8)</th>
<th>Cell count/g after 12 months (×10^8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Maltodextrin</td>
<td>6.3</td>
<td>190.5</td>
<td>4.1</td>
<td>4.2</td>
<td>0.21</td>
<td>0.085</td>
<td>0.015</td>
</tr>
<tr>
<td>2</td>
<td>Calcium gluconate</td>
<td>6.9</td>
<td>195.5</td>
<td>6.9</td>
<td>5.6</td>
<td>4.8</td>
<td>4.3</td>
<td>0.36</td>
</tr>
<tr>
<td>3</td>
<td>Spirulina</td>
<td>7.5</td>
<td>195.5</td>
<td>8.2</td>
<td>6.9</td>
<td>5.7</td>
<td>5.1</td>
<td>0.53</td>
</tr>
<tr>
<td>4</td>
<td>Calcium lactate</td>
<td>8.5</td>
<td>215.0</td>
<td>23.5</td>
<td>7.7</td>
<td>6.9</td>
<td>6.5</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Spray drying parameters: Air inlet temperature 180±1°C, air pressure 2.5kg/cm², air outlet temperature 90±1°C and material flow rate @25 mL/min.

Table 2 — Percentage survival of probiotics as a function of protectant chemicals

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Protectant used</th>
<th>Probiotic species</th>
<th>Percent(w/w)</th>
<th>Method of drying</th>
<th>Survival %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RSM</td>
<td>B. animalis (ssp. animalis DSMZ 20104)</td>
<td>20</td>
<td>Spray drying</td>
<td>87.0</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>RSM</td>
<td>B. animalis (ssp. lactis BB12)</td>
<td>20</td>
<td>Spray drying</td>
<td>79.0</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>RSM + gum acacia</td>
<td>B. animalis (ssp. lactis BB12)</td>
<td>10</td>
<td>Spray drying</td>
<td>72.0</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>Trehalose</td>
<td>L. plantarum 931</td>
<td>10</td>
<td>Convective</td>
<td>54.0</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>Sodium chloride</td>
<td>L. paracasei (NFBC 338)</td>
<td>17.6</td>
<td>Spray drying</td>
<td>30-40</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>Trehalose</td>
<td>L. plantarum (931)</td>
<td>10</td>
<td>Infra-red</td>
<td>3.9</td>
<td>23</td>
</tr>
</tbody>
</table>

soothing properties for enzymes stabilization, but to no avail. Similarly, calcium gluconate was used to impart protection to cell membrane by virtue of Ca²⁺ and harmless attributes of gluconate. However, its performance was only slightly better (0.52% survival) than that of maltodextrin. The choice of Spirulina was due to its fame of a rich source of several vitamins, nutraceuticals, trace minerals, omega-3-fatty acid, among several other health promoting ingredients. The probiotic preparation spray dried with spirulina received dark green colour due to the presence of chlorophyll, whereas finished products with other protectants provided off-white appearance. However, it too gave dismal (0.71% survival) performance.

On this background, calcium lactate has shown 73% survival of spores of Bacillus coagulans. Calcium lactate is produced through microbial fermentation of sugars catalyzed principally by Lactobacillus species. It is deemed GRAS (Generally Recognized As Safe) entity, being used as a feed supplement, food additive, food preservative, pharmaceutical as a calcium replenisher and an intermediate for the commercial production of biodegradable plastics & other “green” compounds.

The reason for the decrease in spore count in maltodextrin, calcium gluconate and Spirulina may be due to less moisture in these materials as compared to calcium lactate (Table 1). Calcium lactate has the property to hold about 23-30% water. Due to this property, calcium lactate has imparted more weight gain to the finished product, which in the absence of any explicable reason, presumably appears to be the most likely reason to promote 73% spore survival.

From Table 2, it is clear that (i) using trehalose, convective current gave 54% survival as against only 3.9% by infra-red exposure, (ii) sodium chloride did not improve % survival, (iii) using RSM alone as a protectant and spray drying method, % survival was 79-87% and (iv) addition of gum acacia did not improve % survival. The % viability of Bacillus coagulans using calcium lactate is comparable with that of B. animalis using RSM due to spray drying and removal of technological hurdles.

Conclusion

From the above studies, it is concluded that calcium lactate has provided (i) higher recovery due to inherent water holding, (ii) superior shelf-life and (iii) increase in nutritional value due to calcium and lactate ions. It has obviously helped to protect cellular components during spray drying and prevented abrupt water desiccation, thereby minimized the shock to Lactobacillus coagulans cells to maintain its cell bound water, rendering less damage to the surface proteins, cell wall, cell membrane and the functional integrity.

Acknowledgement

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