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Research Article

Effect of $NaHCO_3$, $MgSO_4$, Sodium Ascorbate, Sodium Glutamate, Phosphate Buffer on Survival of *Lactobacillus bulgaricus* During Freeze-drying

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Abstract: In the present study, the experiments were investigated the effects of different concentrations of cryoprotective agents, such as $NaHCO_3$, $MgSO_4$, sodium ascorbate, sodium glutamate, phosphate buffer, respectively, which used on survival of *Lactobacillus bulgaricus* during freeze drying. The number of viable cells and survival ratio were measured by the plate count method. The results were as follows: cryoprotective agents played important roles in survival of *Lactobacillus bulgaricus* during freeze drying. When the relative volume of phosphate buffer was 1.5 (v/v), the number of viable cells was highest, while the survival ratio reached highest, the concentration of sodium ascorbate was 4.5%.

Keywords: Cryoprotective agents, freeze drying, *Lactobacillus bulgaricus,* survival ratio, viable cells

INTRODUCTION

During recent years, incorporating probiotic bacteria into dairy foods and developing dried formulations for nutraceutical applications attract more and more attention (Siaterlis *et al*., 2009). For application, probiotic strains growing to high cell densities in the fermentation medium, surviving during freeze drying, remaining viable during storage are important (Antonis *et al*., 2010). So developing technologies are difficulties researchers must solve, which enhance the fermentation productivities and improve cell survival during drying and storage.

Freeze drying damages to biological systems, because it changes in the physical state of the membrane and structure of sensitive proteins in the cell (Leslie *et al*., 1995). In order to improve cell survival, a lot of cryoprotective agents were used, such as disaccharides, polyalcohols, amino acids and proteins (Carvalho *et al*., 2004; Hubalek, 2003). Disaccharides, such as trehalose and sucrose, are good cryoprotective agents, reduce cryo-and thermal injuries and membrane damage during freeze drying and form sugar glass matrices and prevent oxidation during storage (C. Santivarangkna *et al*., 2008).

The aim of this study was to evaluate the effects of cryoprotective agents on the number of viable cells and survival ratio of *Lactobacillus bulgaricus* during freeze-drying. The cryoprotective agents were $NaHCO_3$, $MgSO_4$, sodium ascorbate, sodium glutamate and phosphate buffer. Different concentrations had designed to evaluate protection during freeze drying. The research of sigle protectant provided the experimental basis for the optimization of composite cryoprotective agents.

MATERIALS AND METHODS

Strain and culture conditions: *Lactobacillus bulgaricus* which was obtained from College of Life Science and Engineering, Shaanxi University of Science and Technology was used in the study. *Lactobacillus bulgaricus* was inoculated with 4 % (v/v) inoculum in MRS medium for approximately 18 h at 37°C. After incubation, the culture was centrifuged for 10 min at 3000 g, then discarded the supernatant and re-suspended the cells in an equal volume of Phosphate Buffer Saline (PBS).

Freeze drying: Using a variety of cryoprotective agents, at different concentrations, including $NaHCO_3$, $MgSO_4$, sodium ascorbate, sodium glutamate when the cells were freeze dried. The centrifuged cells were re-suspended in drying medium. The suspensions were incubated at 37°C for 1h ,then frozen at-40°C for 12-24 h and finally freeze dried for 24 h. The dried powder was re-suspended with sterile distilled water and then measured the cell concentration when pre- and post-freeze-drying by viable cell counting. The survival was estimated by: survival = viable cells before drying/viable cells after drying×100%. The powder viable cells = total number of viable cells lyophilized×bacterial suspension volume/power weight.

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Cell concentration: The plate count method was used to measure the number of viable bacterial cells. The culture was diluted in sterile distilled water, then 100 μL of the suspension was spread onto a MRS agar plate. The plates were incubated for about 2 days at 37°C, finally counted them and expressed as Colony Forming Units (CFU) per gramme.

RESULTS AND DISCUSSION

Effect of $NaHCO_3$ on survival of *Lactobacillus bulgaricus* during freeze drying: Effects of different concentration of $NaHCO_3$ on the survival of *Lactobacillus bulgaricus* during freeze drying showed in Fig. 1. When the concentration of $NaHCO_3$ was increased from 0.2% to 0.8%, the number of viable cells and survival ratio increased rapidly and reached the maximum $0.861*10^{11}$cfu/g and 70.6%, respectively. When 0.8% to 1.0%, the number of viable cells and survival ratio reduced rapidly. It demonstrated $NaHCO_3$, when the concentration was 0.8%, offered better protection during freeze drying compared with at other concentration.

Similar observations were reported by Zhenxing *et al*. (2012) for *Lactobacillus acidophilus.* When the $NaHCO_3$ concentrations became 0.4% and 0.6%, the number of viable cells and survival ratio reached the maximum number, respectively. All in all, $NaHCO_3$ was considered a good cryoprotectant for various types of bacteria.

Effect of $MgSO_4$ on survival of *Lactobacillus bulgaricus* during freeze drying: Effects of different concentration of $MgSO_4$ on the survival of *Lactobacillus bulgaricus* during freeze drying showed in Fig. 2. With the concentration of $MgSO_4$ rising from 0.1 to 0.2% and 0.3 to 0.4%, the number of viable cells and survival ratio decreased slowly. During 0.2 to 0.3%, with the concentration of $MgSO_4$ rising, the number of viable cells and survival ratio both increased. The optimal concentration of $MgSO_4$ was 0.5%. The number of viable cells and survival ratio were $0.8*10^{11}$ cfu/g and 76.4%, respectively. A possible reason was that divalent cation ($MgSO_4$) could strengthen preferential exclusion by enhancing the hydrophobic character of the protein (Tan *et al*., 1995).

Effect of sodium ascorbate on survival of *Lactobacillus bulgaricus* during freeze drying: Effects of different concentration of sodium ascorbate on the survival of *Lactobacillus bulgaricus* during freeze drying showed in Fig. 3. During 3 to 4.5%, with the concentration of sodium ascorbate rising, the number of viable cells and survival ratio both increased, but during 4.5 to 6.0%, those both reduced. Over 6.0%, the number of viable cells and survival ratio both increased rapidly and were 0.51×10^{11} cfu/g and 84.7%. The optimal concentration of sodium ascorbate

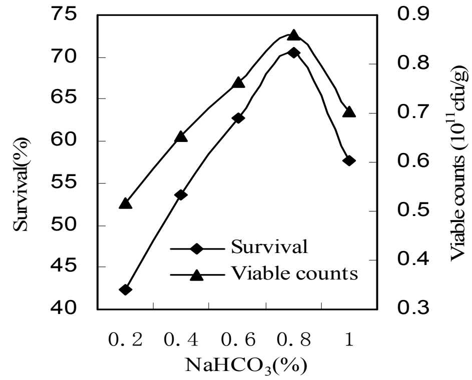

Fig. 1: Effect of $NaHCO_3$ on the survival of *Lactobacillus bulgaricus*

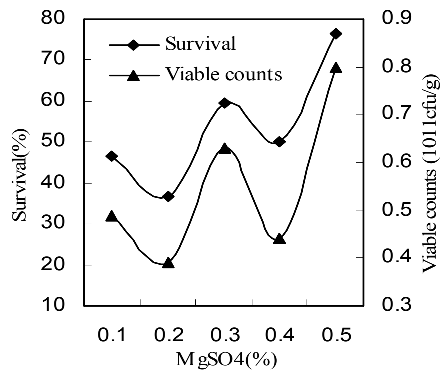

Fig. 2: Effect of NgSO4 on the survival of Lactobacillus bulgaricus

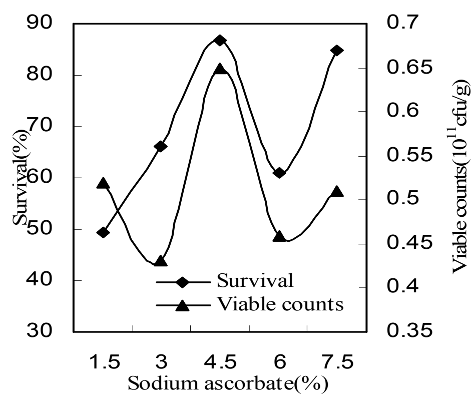

Fig. 3: Effect of sodium ascorbate on the survival of *Lactobacillus bulgaricus*

was 4.5%. They were $0.65*10^{11}$ cfu/g and 86.8%, respectively.

Kurtmann *et al.* (2009) showed that presence of sodium ascorbate increased storage stability of freeze-dried *Lactobacillus acidophilus* and concluded that sodium ascorbate was an important factor influencing the survival of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* during freeze drying and storage.

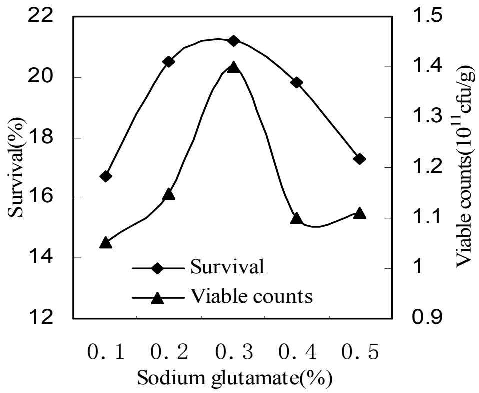

Fig. 4: Effect of sodium glutamate on the survival of *Lactobacillus bulgaricus*

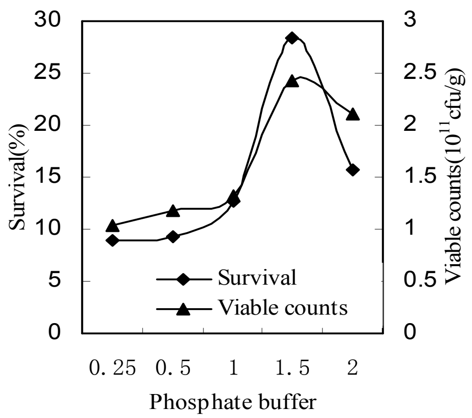

Fig. 5: Effect of phosphate buffer on the survival of *Lactobacillus bulgaricus*

Effect of sodium glutamate on survival of *Lactobacillus bulgaricus* during freeze drying: Effects of different concentration of sodium glutamate on the survival of *Lactobacillus bulgaricus* during freeze drying showed in Fig. 4. With the concentration of sodium glutamate rising from 0.1 to 0.3%, the number of viable cells and survival ratio both increased and reached maximum, $1.4*10^{11}$cfu/g and 21.2%, respectively. When 0.3 to 0.5%, the number of viable cells and survival ratio both reduced rapidly. In accordance with our results, when the concentration was 0.3%, sodium glutamate offered better protection during freeze drying compared with at other concentration.

Similarly, Abadias *et al.* (2001) reported that sodium glutamate at 10% were the best protective agents used on the viability of the bio control yeast *Candida sake.* It demonstrated, for various types of bacteria, sodium glutamate can offer the protection during freeze drying, but the degree of protection depended on the concentration of sodium glutamate.

Effect of phosphate buffer on survival of *Lactobacillus bulgaricus* during freeze drying: Effects of different relative volume of phosphate buffer on the survival of *Lactobacillus bulgaricus* during freeze drying showed in Fig. 5. The relative volume was estimated by: (v/v) relative volume = v_1/v_2, v_1 was the volume of phosphate buffer; v_2 was the volume of bacterial sludge. When the relative volume was increased from 0.25 to 1.0, the number of viable cells and survival ratio increased slowly, but over 1.0, them increased rapidly and reached the maximum 2.42×10^{11}cfu/g and 28.36% at 1.5, respectively. When 1.5 to 2.0, the number of viable cells and survival ratio reduced rapidly.

CONCLUSION

The choice of the cryoprotectant and its concentration affected the survival of *Lactobacillus bulgaricus* during freeze drying. Addition of NaHCO3, $MgSO_4$ and sodium ascorbate had more important effects on the survival ratio while sodium glutamate and phosphate buffer. But glutamate and phosphate buffer used as protective agent, *Lactobacillus bulgaricus* reached the higher number of viable cells.

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