

Research Article

Improving the Survival of *Arthrobacter* sp., CW9 during Spray Drying Monitored by Scan Electric Microscope

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Abstract: The culture of an aquaculture probiotic, i.e., *Arthrobacter* sp., CW9, was spray dried with different carriers/protectants, in which Scan Electric Microscope (SEM) was used to analyze the surface of micro-particles produced by spray-drying. Matrix of protectants, inlet temperature and feed rate were optimized according to the survival rate after spray drying. Scanning electron micrographs showed that cracks formed on the particle surface were a key factor in enhancing bacteria survival during spray-drying. Span-60 had synergism with Skim Milk Powder (SMP): trehalose (7.5%: 7.5%, w/v) in protecting *Arthrobacter* sp., CW9 bacteria from heat injury, unlike SMP with trehalose. Particle size is an important factor influencing bacteria survival during spray drying and particle size itself was influenced by certain additives.

Keywords: Bioprocessing, dairy, probiotics

INTRODUCTION

During recent decades, there has been increased interest in probiotic foods due to consumer interest in health benefits. The health benefits associated with probiotic food products are based on the presence of selected viable strains of microorganisms which, when administered in adequate amounts, improve the health of the host (Rubio *et al.*, 2013). Therefore, in addition to the requirements for good safety and functional characteristics, probiotic cultures should be able to withstand food processing and storage conditions encountered during the manufacture of functional foods under industrial conditions (Desmonda *et al.*, 2011).

A major challenge associated with the application of probiotic cultures in functional foods is the retention of viability during processing. Among varying processing means, spray drying is a widely used industrial-scale process in drying probiotic cultures due to the very short drying time, the ability to obtain a powder form product, less expensive and more energy-efficient (Ananta *et al.*, 2004; Filkova *et al.*, 1997).

During spray drying of probiotic culture, a suitable carrier medium is essential and reconstituted skim-milk powder would appear to be a suitable carrier medium for an efficient spray drying of probiotic cultures and a wide variety of carriers, including whey protein, trehalose, mono-sodium glutamate, gum acacia,

glycerol, betaine, adonitol, sucrose, glucose, inulin, lactose and oligo-saccharides, have been used as protectants for improving the survival of bacteria during this process (Ananta *et al.*, 2004, 2005; Gardiner *et al.*, 2000; Golowczyc *et al.*, 2011; Sunny-Roberts and Knorr, 2009). Some groups have investigated the mechanism of these protectants on enhancing probiotic survival during drying (Crowe *et al.*, 1996; Schwab *et al.*, 2007; Sunny-Roberts and Knorr, 2009).

In this study, Scan Electric Microscope (SEM) was used for the first time to analyze the surface of microparticles produced by spray-drying a mixture suspension of several protectants and the culture of an aquaculture probiotic *Arthrobacter* sp., CW9, to give an insight into the effect of different protectants on the bacteria survival rate.

MATERIALS AND METHODS

Organism: *Arthrobacter* sp., CW9 (CGMCC No. 4197) was originally isolated from aquatic water in Lianyungang, Jiangsu Province, China and preserved in 20% glycerin at -20°C.

Preculture and production of cell concentrates: *Arthrobacter* sp., CW9 was grown in 250 mL shaker flasks containing 50 mL DPYN medium (8 g dextrose/L, 5 g peptone/L, 3 g yeast extract/L, 2.0 g

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NaCl/L, pH 7.0). The preculture was inoculated with two loops of overnight culture from a slant and incubated at 27°C and 180 rpm for 16 h. This preculture was then used to inoculate a 16 L Bioengineering AG L1523 fermenter (5% v/v) with a working volume of 12 L. The fermenter was incubated for 18 h at 27°C, 300 rev/min and 80 L/h of air feeding. The pH was controlled constantly at 7.0 by feeding with either 2 M NaOH or 2 M HCl. Initial viable cell concentrations of *Arthrobacter* sp., CW9 in fermentation broth were determined by plating on DPYN agar at 27°C for 24 h. Results were expressed in colony forming units (cfu) mL⁻¹.

Effect of spray-drying on bacterial survival: Spray-dried powders were prepared under the following standard conditions unless specific experimental conditions are described. The cells were harvested by centrifugation for 10 min at 5,000×g and washed twice by centrifuging with sterilizing saline. The cellular pellet was re-suspended in different protectant solutions, which was 1/4 of the original volume of the fermentation broth. The suspension was spray dried in a laboratory-scale spray dryer (SP-1500, Shanghai Shunyi Tech. Co., Ltd.) with an inlet temperature of 100°C and outlet temperature of 58-59°C (room temperature = 5°C) at a feed rate of 400 mL/h. Dried powder samples were stored in amber glasses hermetically closed at 4°C until further use. Additional triplicate suspension samples (10 mL) and dry powder (1 g) were dried to constant weight at 105°C in a convection oven to get the dry matter in the initial suspension and the moisture content of the dry powder. The percentage of powder recovery after spray drying was calculated by taking the difference between the final weight of the powder in the collector bottle and the weight of the solids in the initial suspension.

To determine bacterial survival after spray-drying, triplicate samples of each spray-dried powder (0.1 g) were rehydrated with 5 mL DPYN medium. Samples were shaken vigorously for 1 min and then allowed to rehydrate for 9 min before their viable cell concentrations (cfu/g) were determined by plating on DPYN agar. The percentage of surviving cells was then calculated.

SEM: The powder samples were mounted on aluminum stubs using double-sided adhesive tape. Excess particles were removed by directing a jet of dry air at the surface of the stub. The samples were then coated with gold in a sputter coater and were examined with a JSM-6390LA SEM (JEOL Ltd., Japan) operating at an accelerating voltage of 15 kV.

RESULTS AND DISCUSSION

Protectants: Six materials, namely Skim Milk Powder (SMP), Gelatin (GL), Carrageenan (CR), Sucrose (SC), Trehalose (THL) and Monosodium Glutamate (MSG), were selected as protectants for spray drying of *Arthrobacter* sp., CW9. The concentration and effects of these protectants are shown in Fig. 1 and 2 shows the scanning electron micrographs of the spray-dried powder prepared with different protectants.

SMP showed the best protection effect among these protectants, secondly THL. The particle size formed by spray drying employing SMP, GL, CR and MSG were much smaller than that of SC and THL (Fig. 2). Although the particles of both SC and THL were similar agglomerates, there are many cracks on the surface of the particles of THL, indicating that the

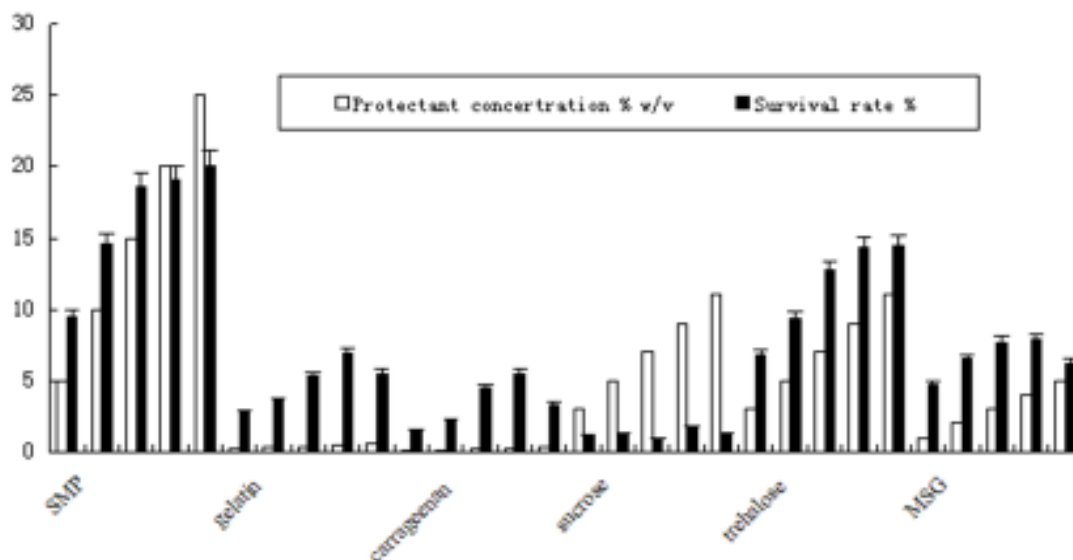


Fig. 1: Survival rate of *Arthrobacter* sp., CW9 spray dried with different protectants

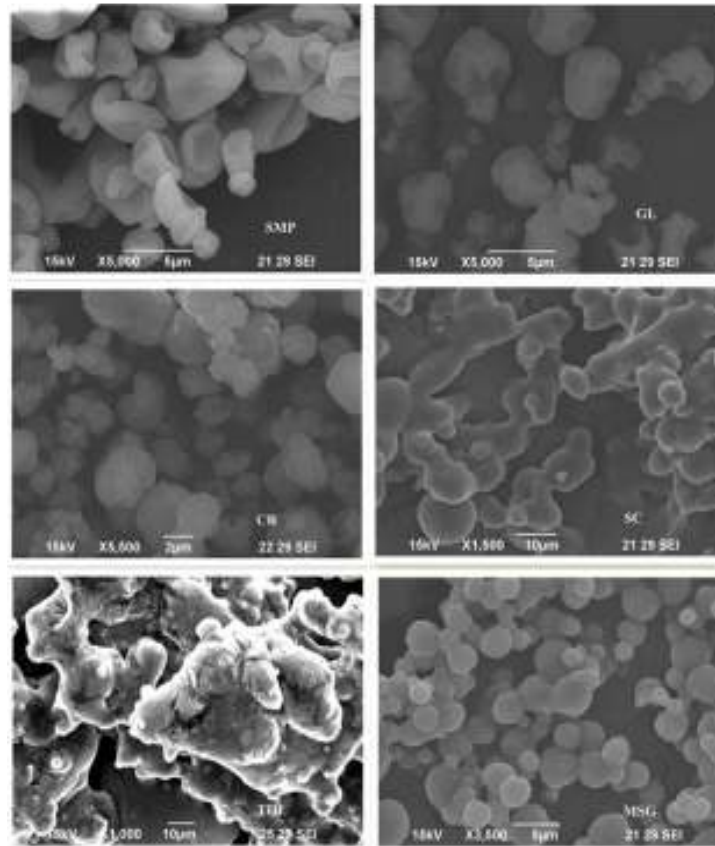


Fig. 2: Scanning electron micrographs of the spray-dried powder prepared with different protectants
 SMP: Skim milk powder; GL: Gelatin, CR: Carrageenan; SC: Sucrose; THL: Trehalose; MSG: Monosodium glutamate

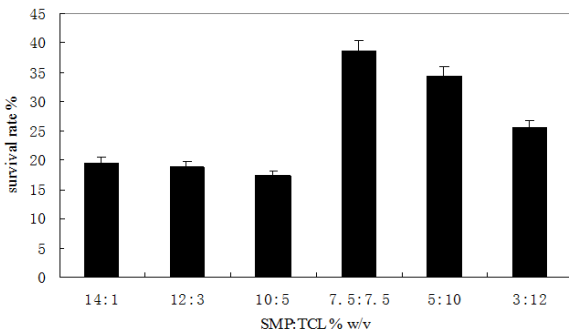


Fig. 3: Survival rate of *Arthrobacter* sp., CW9 spray dried with SMP and trehalose

bacterial survival are affected by the particle size, the cracks on the particle surface and the protectant type. Similarly, the protecting effects of different components of SMP on bioactive have been reported by Livney (2010). In addition, it is also known that intracellular trehalose provides thermal stability for yeast cells (Hottiger *et al.*, 1989). Therefore, the combined effect of SMP and THL was selected for the further study.

SMP and THL: Part of SMP was replaced with THL as carrier in spray drying process. The bacteria survival

rates were shown in Fig. 3 and the scanning electron micrographs was shown in Fig. 4. With the increase of the percentage of THL, bacteria survival decreased first, increased sharply when half of the SMP was replaced by THL and then decreased again. The size of the particles changed little while there were distinct changes on the surface. When not more than 1/3 of SMP was replaced with THL, the particle surface was shriveled. In the case of 1/2 of SMP was replaced with THL, the particles were round and cracks occurred on the particle surface. And both the quantity and the width of cracks increased with the increase of the ratio of THL. Drying of the droplets by hot air in spray driers takes place in two steps, i.e., the constant rate period and falling rate period (Birchal *et al.*, 2005). During the second period, the droplets became hollow particles with a solid SMP crust formed at their surface and vapor inside. The crust thickened and prevented vapour from diffusing outside. As a consequence, the particle starts to heat up. We can deduce that the major thermally injury on bacteria happened during this period. When hollow particles cooled down, the vapor inside condensed and the hollow particles deflated and shriveled (Birchal *et al.*, 2005) and pets form on the surface. While more than 1/3 of SMP was replaced with trehalose, cracks formed on the particle surface enabled the vapour inside to diffuse out continuously and,

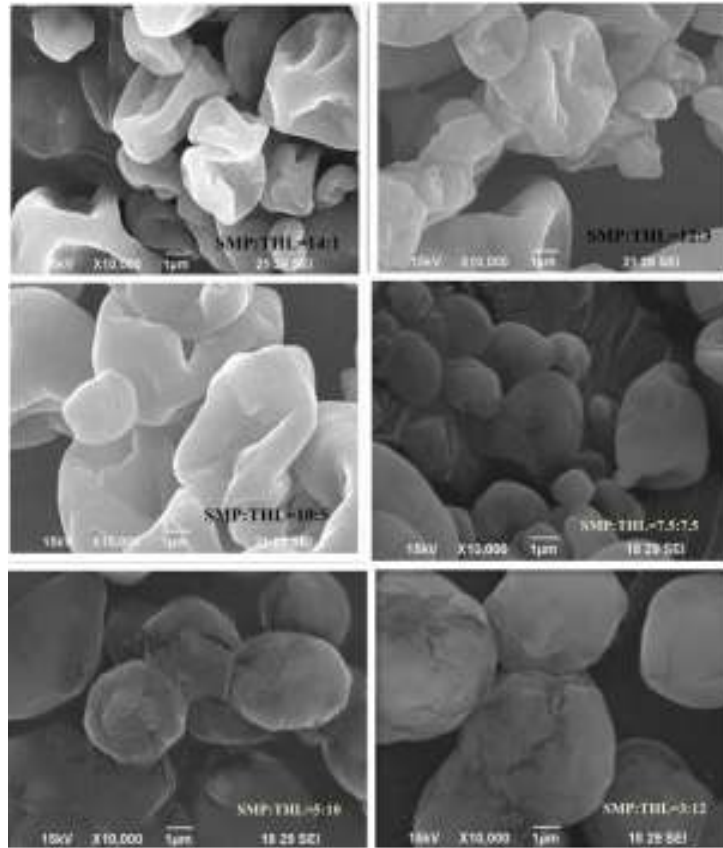


Fig. 4: Scanning electron micrographs of the spray-dried powder prepared with SMP and THL

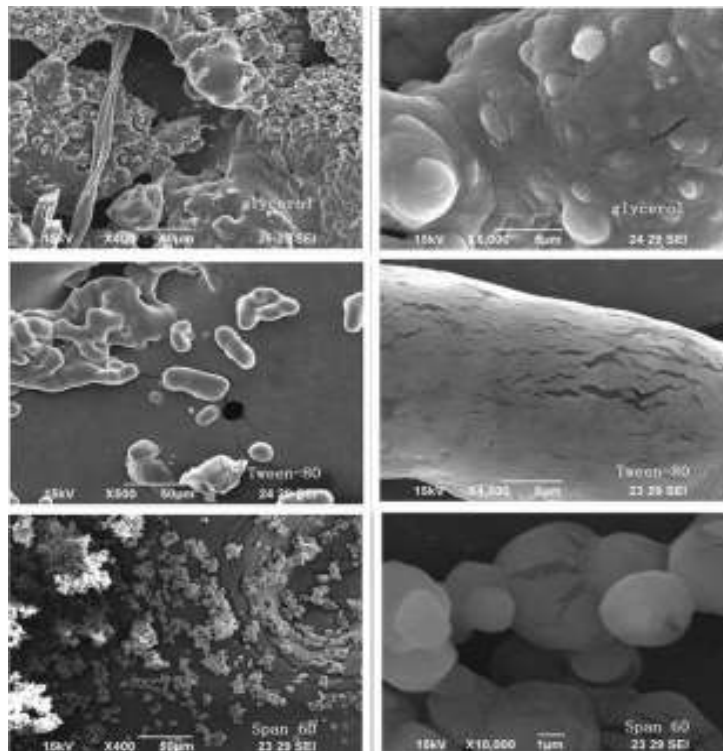


Fig. 5: Scanning electron micrographs of the spray-dried powder prepared with glycerol, tween-80 and span-60 in addition to SMP-THL combination

consequently, particles temperature keeps constant or rises up very slower, so the injury on bacteria in particles decreased and the particles keep round finally. This phenomenon was stated previously by Lian *et al.* (2002), but where only skim milk (10%) was used as carrier. Besides, the decrease of bacteria availability at low trehalose concentration indicated that SMP has no synergistic action with trehalose on protecting the bacteria in spray drying process.

Glycerol, tween-80 and span-60: Glycerol, Tween-80 and Span-60 were added into SMP-THL (7.5%: 7.5%, w/v) protectant solution to re-suspend centrifugal cellular pellet. All three additives had synergism with SMP-THL (7.5%: 7.5%, w/v) and glycerol was the best. But the powder produced with glycerol was too sticky to harvest from the harvesting bottle and the cyclone wall and easy to moisture as well. Thus make it difficult to apply. Figure 5 shows scanning electron micrographs of the spray-dried powder prepared with different protectants. Scanning electron micrographs show that all particle' surfaces are rounded but filiform structures can be seen in powder spray dried with glycerol. The size of particles with Tween-80 enlarged significantly while the size of particles with Span-60 remained unchanged. Obviously, particle size is an important factor influencing bacteria survival during spray drying. The size of the dried particle in SMP spray drying was often used for estimating droplet size of a spray, assuming that only uniform shrinkage or vacuole formation has taken place (Dijck *et al.*, 1995; Kim *et al.*, 2009). But here we see that the size of the dried particle might change a lot while certain additives exist, although droplet sizes leaving atomizer were uniform.

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