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Heat stability of *Lactobacillus rhamnosus* GG and its cellular membrane during droplet drying and heat treatment

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ABSTRACT

Dehydration and thermal stresses are generally considered as two mains factors deactivating probiotic cells during droplet drying, as typically in industrial spray drying for producing active dry probiotics. However, little is known about how cells respond to these interplaying stresses in the short period of drying. This study showed that dehydration process could alleviate the detrimental effect of thermal stress to a certain extent, evidenced by that probiotic cells could withstand higher temperature in a single droplet drying (SDD) process compared to sole heat treatment. During SDD at 90 °C, droplet temperature increased with time, and the inactivation of *Lactobacillus rhamnosus* GG (LGG) was initially observed at droplet temperature of 61–65 °C. By contrast, the transition from the maintenance of LGG viability to rapid deactivation occurred at around 54 °C in heat treatment without dynamic dehydration. Possible mechanisms for the enhanced thermotolerance were investigated from drying kinetics level and cellular level. The favorable temperature profile and the decrease in droplet water activity during drying may benefit cell survival. The cytoplasmic membrane of LGG was more stable at elevated temperatures of 60–65 °C during drying, which might be related to the high viscosity of semi-dried particles mitigating the leakage of intracellular substances. Trehalose demonstrated a strong thermoprotective effect over lactose in heat treatment, but the protection was less effective at the later stage of drying. These results dissected the influence of the interplaying stresses on probiotic cells for the first time during droplet drying and also suggested possible approaches for improving cell survival in dried particles. Components capable of protecting cellular membrane are recommended for developing protectant formulation in spray drying of probiotics.

1. Introduction

Spray drying offers a cost-effective alternative to freeze drying for producing active dry probiotics in a powder form that is ready to be incorporated in food or pharmaceutical products (Fu & Chen, 2011; Schutyser, Perdana, & Boom, 2012). How to preserve cell viability during spray drying has attracted growing attention in recent years, as the harsh drying conditions may cause up to several logs reduction in cell population (Khem, Bansal, Small, & May, 2016; Liu et al., 2015; Salar-Behzadi et al., 2013). The survival of probiotics in dried particles is closely related to droplet drying kinetics experienced by the cells, such as changes in droplet temperature (T_d), moisture content (X), rate of temperature change (dT/dt), and rate of moisture content change (drying rate, $-dX/dt$) (Chen & Patel, 2007; Li, Lin, Chen, Chen, & Pearce, 2006). Droplet drying kinetics are influenced by both droplet properties and air conditions, including the size and solids content of droplets, the property of solids, air temperature, air flow rate and humidity (Fu, Woo, Lin, Zhou, & Chen, 2011). As such, drying kinetics may vary between different dryers even with the same operation settings, depending on the type of atomizer and air dynamics of the dryer. To generalize the preservation of probiotics for all types of spray dryers, it is crucial to understand the mechanisms for maintaining cell viability during drying, which include but are not limited to: response of cells to droplet drying kinetics, interactions between probiotic cells and protectant solids, critical cell structures that are responsible for cell deactivation, influence of each environmental stress on cellular structures, and possible approaches to mitigate the detrimental effects of these stresses.

During spray drying, atomized droplets is rapidly dehydrated in a hot air stream. The simultaneous heating and dehydration lead to continuously changing droplet temperature and moisture content, which may impact bacterial viability in a different way from heat stress

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at a constant temperature, as in conventional heat treatment without drying involved (Chen & Patel, 2007). A couple of studies suggested that at lower droplet temperature (< 45–55 °C), dehydration inactivation was the main cause of cell death, which depended either on the moisture content or on the drying rate; by contrast, at higher droplet temperature (> 45–65 °C), cells were inactivated by a combination of thermal and dehydration stresses (Ghandi, Powell, Chen, & Adhikari, 2012; Perdana et al., 2013). However, how changes in droplet moisture content and drying rate affect the survival of bacterial cells has not been substantiated.

The temperature range of 45–65 °C is around or slightly lower than the outlet temperature range commonly adopted in spray drying studies to produce active dry probiotics (Gardiner et al., 2002; Huang et al., 2017). In such a process, probiotic cells may suffer from several stresses such as heat, osmotic, oxidative, and other stresses. These stresses can induce injuries on multiple cellular sites such as cytoplasmic membrane, ribosomes and chromosome, leading to the deactivation of cells (Fu, Woo, Selomulya, & Chen, 2013; Khem, Woo, Small, Chen, & May, 2015). Owing to the structure of spray dryers, it is relatively difficult to monitor the changes of atomized droplets during drying (Fu et al., 2017; Ghandi et al., 2012; Triboli & Gut, 2016). In reported studies, the injuries of probiotic cells were often studied after spray drying, to examine the effect of drying conditions or the effect of protectant composition on cellular structures (Golowczyc, Silva, Teixeira, De Antoni, & Abraham, 2011; Iaconelli et al., 2015; Liu et al., 2015). However, this kind of end-point analysis could not provide insights on the effect of drying kinetics, for example the rate of temperature change and drying rate, which impact cell survival in both heat treatment and drying processes (Khem et al., 2015; Marechal, Martínez de Marnañón, Poirier, & Gervais, 1999). Salar-Behzadi et al. (2013) examined changes in the viability, cell membrane and esterase activity of two probiotic strains as heat treatment progressed, and the results were used to interpret observations in spray drying process. Their study clearly showed the progressive changes of cellular properties in response to heat stress, but there was no experimental analysis about the dehydration effect, which occurs simultaneously with heating during spray drying.

Experimental investigations on in-drying physicochemical changes associated with spray drying are often performed with the single droplet drying (SDD) technique (Fu, Woo, & Chen, 2012; Schutyser et al., 2012). Its principle is to dry a tiny droplet under controlled air conditions analogous to spray drying and to monitor the kinetic and physicochemical changes of the droplet as drying progresses. The SDD technique has been applied to study the inactivation profiles of probiotics as influenced by drying kinetics and protectant composition (Fu et al., 2013; Ghandi et al., 2012; Perdana, Fox, Siwei, Boom, & Schutyser, 2014; Zheng et al., 2015). In the present study, it was utilized to monitor the changes in the viability and cellular membrane of a model probiotic strain, *Lactobacillus rhamnosus* GG (LGG), in two types of protectants. The trends were compared to control experiments with heat treatment, to investigate the research problem: how dehydration stress and thermal stress influence probiotic cells during droplet drying. Two hypotheses were tested: (1) compared to heat treatment, the additional dehydration stress during droplet drying would cause a more rapid inactivation and more severe cellular injuries of LGG cells; (2) trehalose, which is a prestigious protectant against dehydration stress (Perdana et al., 2014; Tymczyszyn, Gomez-Zavaglia, & Disalvo, 2007), would help alleviate the dehydration stress during droplet drying and hence effectively protect the viability and cellular structures of LGG cells. Lactose was used as another protectant, because it was known for insignificant protection compared to trehalose and other milk components (Zheng et al., 2015; Zheng, Fu, Huang, Jeantet, & Chen, 2016).

2. Material and methods

2.1. Microorganism

Lactobacillus rhamnosus GG (LGG) was obtained from CVS Pharmacy (Culturelle®, New Haven, USA). The culture was maintained on MRS agar plates at 4 °C with subculture to fresh medium every seven days. Bacterial cells were grown on MRS agar plates (Oxoid Ltd., Hampshire, UK) at 37 °C for 48 h.

2.2. Preparation of cell suspension for heat treatment and drying experiments

Inoculum was prepared by aseptically transferring a single colony of LGG to 10 mL MRS broth with subsequent incubation at 37 °C for 12 h. Then, 100 mL MRS broth was inoculated with 1% (v/v) inoculum size and incubated at 37 °C for 24 h. The viable cell concentration in the 24-h culture was 1–2 × 10^9 cfu/mL. Cells were collected by centrifugation at 10000*g*, 4 °C, for 10 min. The collected cell pellets were washed twice using 0.5% (*w/v*) peptone solution and centrifuged. Then, a protectant solution was added to the centrifuge tube to re-suspend the cell pellet; the volume of the protectant was controlled to ensure that the final suspension had the same cell concentration to the 24-h culture.

Two types of protectant were utilized, i.e., 10.0% (w/w) lactose (Sigma Aldrich, Shanghai, China) and 10.0% (w/w) trehalose (BioDee, Beijing, China) solutions. The lactose solution was autoclaved at 105 °C for 10 min and trehalose solution was sterilized at 121 °C for 15 min. For each heat treatment experiment, six samples of 6 mL were prepared for each protectant in 10 mL round-bottomed centrifuge tubes, corresponding to heat treatment time of 0, 30, 60, 90, 120 and 240 s. The use of 6 mL for each sample was to ensure sufficient sample amount for subsequent cellular injury analyses. For SDD experiment, 1 mL of sample was prepared following a reported procedure (Zheng et al., 2016). All cell suspensions were prepared freshly on the day of drying or heat treatment experiment, and used for the experiment immediately after preparation.

2.3. Single droplet drying (SDD) experiment

Detailed procedure of SDD experiments has been reported previously (Fu et al., 2011; Zheng et al., 2015). The SDD apparatus (SDPA MARK II, Nantong Dong-Concept New Material Technology Ltd., Jiangsu, China) was capable of generating stable air flow with controlled temperature, velocity and humidity to dry an isolated droplet in a confined drying chamber (Zheng et al., 2016). Airflow was generated from a compressor, and passed through a set of filters, a dehumidifier, a heating column and distributing meshes to ensure its stability in the drying chamber. The droplet was suspended at the tip of a specially made glass filament and the hot air went upwards to dry the droplet. The apparatus was equipped with three droplet suspension modules, used for droplet temperature, diameter and mass measurements, respectively. In the present study, the diameter-measuring module was used to suspend the protectant droplets containing LGG to determine the loss of viability as drying progressed. Droplet temperature was measured with the temperature-measuring module at separated drying runs with identical conditions. Changes in droplet mass was monitored by the deflection of a mass-measuring glass filament following a reported procedure (Fu et al., 2011). Drying conditions used in the present study were: air temperature of 90 °C, velocity of 0.75 m/s, humidity of 0.001 kg/kg (corresponding to relative humidity of 0.25% at 90 °C), and initial droplet size of 2 μL.

To monitor changes in LGG viability during drying, SDD runs were stopped at different stages (0, 90, 105, 120, 135, 150, 165 and 240 s), and the semi-dried particles were dissolved in situ in 2 mL of peptone solution. Then, the standard plate count analysis was performed on MRS agar to enumerate residual viable cells in each sample. The