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A COMPARISON OF THE SURVIVAL RATES OF E. COLI K12 AND L. ACIDOPHILUS IN SPRAY DRYING

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Abstract: The survival of mid-exponential and the early-stationary E. coli K12 and L. acidophilus were investigated when spray drying and outlet air temperatures of 60, 70, 80, 90 and 100°C. The results showed that the early-stationary cell of both cultures had a greater heat resistance than the mid-log cell in every drying temperature. The best survival rate was found when spray drying at temperature lower 80°C and it is showed that L. acidophilus is stronger than E. coli K12 (irrespective of the growth phase).

Keywords: L. acidophilus, E. coli K12, spray drying

INTRODUCTION

In food or pharmaceutical manufacturing, drying is a useful technique to preserve raw materials as well as to fabricate products. Although there are several techniques that can produce dried microbial cells, spray drying is the most common technique for drying bacteria cell because of its lower cost and high capacity, which makes it suitable for large-scale production. This technique typically produces about 10% water content of dried particle from a feed solution containing 40-60% water content (Heldman and Hartel, 1997). However, the high temperatures encountered during processing may cause cell injury. There are many factors that affect the survival rate of cells in drying treatments, e.g. bacterial strain, drying medium, growth media, growth phase and cell concentration, spray drying condition, rehydration conditions and storage and packaging conditions (Meng et al., 2008; Morgan et al., 2006; Lian et al., 2002; Rodriguez-Hueso et al., 2007;Corcoran et al., 2004). Considering the effects of spray drying temperature on survival rate of cell bacteria, many workers have reported decreased numbers of viable cells when using high temperature, and this is attributed to the fracture of particles (Lian et al., 2002; Rodriguez-Hueso et al., 2007;Corcoran et al., 2004).

Bacterial cell walls principally consist of peptidoglycan, which is the main component that governs the shape of bacteria, but also include other polymers that vary in different type of bacteria. Bacteria are generally divided into two categories depending on the type of cell wall: Gram positive and Gram negative bacteria (Heritage et al., 1996; Hammond et al., 1984). Gram positive cell walls consists of almost 50% peptidoglycan. Other components include teichoic acids, teichuronic acids and proteins. The thick layer characteristic of Gram positive cell walls can protect cell from harsh environment such as heat and pressure stress. For example, it is claimed that the cell walls of some strains of Gram positive bacteria are able to resist a high pressure up to 200 atmospheres (Heritage et al., 1996).

In contrast with Gram positive bacteria, the cell walls of Gram negative bacteria are more complex, comprising a lower proportion of peptidoglycan (10-20 % of weight of cell wall), but also protein, lipoprotein and lipopolysaccharide that is located at outer membrane. The outer membrane in Gram negative cells acts as the barrier to protect the cell from the environment, and helps cells to survive and grow in adverse environment conditions, for example, in digestive tract that occupied bile salt and hydrolyzed enzyme. In addition, because of the cell wall features, it is impermeable to hydrophobic and high molecular weight compounds. This guards the cell from antibiotics and antimetabolites as well as some lethal agents (Heritage et al., 1996).

In the spray drying process, selecting the drying air conditions should consider the individual heat tolerances of bacterial strains. Differences in cell wall components between Gram negative bacteria (E. coli K12) and Gram positive bacteria (L. acidophilus) may affect the survival rate of cells during the drying process. In addition, in each stage of population growth of bacteria, cells have a difference physiology that could affect heat tolerance. The main questions we wish to investigate is how does the growth phase (mid-log or early stationary) effect heat tolerance and how
do Gram negative and Gram positive bacteria compare. In this study, the spray drying of *E. coli K12* (a Gram negative bacterium) and *L. acidophilus* (a Gram positive bacterium) were performed to study the effect of growth phase and spray drying outlet temperature (60, 70, 80, 90 and 100°C) on the cell viability of spray dried cultures.

**MATERIALS AND METHODS**

**Microorganisms**

*E. coli K12* was originally obtained from Prof. T.F. Brocklehurst (Institute of Food Research, Norwich, UK). Cells were activated and grown in tryptone soya broth (TSB; Oxoid, Hampshire, UK) and incubated overnight at 30°C in an orbital incubator. These activated cultures were used for preparation of cell pastes and for preparation of solutions for spray drying.

The freeze dried form of *L. acidophilus NCIMB 70225* was purchased from National Collection Microbial (NCIMB). Cells were activated, transferred into new Man, Rogosa and Sharpe broth (MRS broth; Oxoid, Hampshire, UK) and incubated at 37°C for 72 hours under anaerobic conditions using a Gas Pack system. Broth cultures were kept in a fridge before using as a starter culture for preparations of cell pastes or population growth experiment.

For some experiments cell pastes were prepared by harvesting and centrifuging cells (spin at 3000g, 4°C for 10 minutes) from 1L cultured broth. This was added to 5 mL of its medium broth, and mixed to obtain a homogeneous cell paste. This was then kept in the refrigerator at 4°C before using in spray drying experiments on the same day (method adapted from Chen et al., 2006).

**Population growth study**

**E. coli K12**

Activated cultures were inoculated into individual 100 mL MRS broth in 250 mL Erlenmeyer flask, incubated at 37°C for 24-50 hours under anaerobic condition controlled by Gas pack system. Samples were taken at 1 hour intervals to count cell number in CFU/mL by the pour plate technique on MRS agar (Oxoid, Hampshire, UK), cell mass density by spectrophotometer (OD$_{600}$) and pH of broth culture by Ion Meter (Jenway model 3340, Bibby Scientific, Stone, UK) during cultivation. All experiments were done in duplicate. Population growth curves were built from cell numbers in log$_{10}$ scale plotted versus incubation time.

**Feed solution preparation for spray drying**

Four types of feed solution are defined here depending on whether *E. coli* or *L. acidophilus* was used and whether the cells were centrifuged to form a cell paste (without the broth components), as follows:

- **Feed solution E1**: Cultured broth of *E. coli K12*, 500 mL, either in the mid-log phase or in the early-stationary phase was taken and mixed homogeneously with 20% w/v maltodextrin solution DE 17-19 (myprotein.co.uk, Manchester, UK) by aseptic technique.

- **Feed solution E2**: Cell paste of *E. coli K12*, either in the mid-log phase or in the early-stationary phase, was mixed homogeneously with sterilised 20% w/v maltodextrin solution.

- **Feed solution L1**: Cultured broth of *L. acidophilus*, 500 mL, either in the mid-log phase or in the early-stationary phase was taken and mixed homogeneously with 20% w/v maltodextrin by aseptic technique.

- **Feed solution L2**: Cell paste of *L. acidophilus*, either in the mid-log phase or in the early-stationary phase, was mixed homogeneously with sterilised 20% w/v maltodextrin solution.

Thus, suffix 1 represents directly mixing the cultured nutrient broth with maltodextrin solution, whereas suffix 2 means the broth was first centrifuged to remove broth components and create a cell paste before adding maltodextrin (as described earlier).

**Spray drying study**

Spray drying was performed in a tall-form co-current spray drier of 12 ft height x 4 ft diameter (Spray Processes, Bedford UK). A peristaltic pump (Watson-Marlow 510U) was used to deliver the feed solution to the atomiser. The atomisation was performed by a twin-fluid nozzle, using compressed air as the atomising gas. Ambient air was directly heated in a burner using natural gas,
allowing control of the inlet air temperature. A HEPA filter was used to filter bacteria from the outlet air stream. The operation was started by warming the chamber for 10-15 minutes before feeding distilled water and the inlet and outlet temperatures were set by adjusting the liquid feed and air flow rate. The outlet temperature was effectively regulated by variation of the liquid feed flow rate. Drying outlet temperatures ranging between 60 and 100°C were used (according to Table 1). Once the required outlet temperature was reached, the solution was fed into the drying chamber. followed spray drying conditions 1-4 (see Table 1). The dried powders were collected in a cyclone separator and transferred to a sealed tight sterilized amber bottle, and taken for analysis. All experiments were carried out in duplicate.

Table 1. Spray drying conditions

<table>
<thead>
<tr>
<th>Feed solution</th>
<th>Drying outlet temperatures used (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>60, 70, 80, 90 and 100</td>
</tr>
<tr>
<td>E2</td>
<td>70 and 80</td>
</tr>
<tr>
<td>L1</td>
<td>70 and 80</td>
</tr>
<tr>
<td>L2</td>
<td>60, 70, 80, 90 and 100</td>
</tr>
</tbody>
</table>

Determination of powder moisture content

The moisture content of the spray dried powders was determined according to AOAC method 960.18 (OMA) by oven drying at 105±1°C for 24 hr. The percentage moisture content values were then used to calculate cell survival rates on a dry basis.

Determination of cell numbers and cell survival rate

For the population growth study, culture broths were taken and serially diluted in PBS, and plate counted on TSA (E. coli K12) or MRS agar (L. acidophilus). Data are presented in colony forming units per millilitre (CFU/mL).

In the spray drying study, approximately 2 g. of dried cells were rehydrated in PBS and incubated for 1 hour at 30°C, for E. coli K12, and at 37°C for L. acidophilus (under anaerobic conditions). Then samples were serially diluted with PBS and cells counted by the pour plate method. The number of viable cells was expressed in colony forming units per gramme of dried sample (CFU/g).

The percentage of bacteria surviving spray drying was calculated as follows (Rodríguez-Huezo et al., 2007):

\[
\% \text{ Survival} = \frac{N}{N_0} \times 100 \tag{1}
\]

where

\(N\) is Log cell number of bacteria after treatment (Log CFU/g. dried sample)

\(N_0\) is Log cell number of bacteria before spray drying (Log CFU/g. dried sample)

RESULTS AND DISCUSSION

This present work explored the effect of cell growth phase and drying outlet temperature on cell survival in the spray drying process.

Population growth study

E. coli K12

Fig. 1 shows the results of triplicate experiments of population growth of E. coli K12 in TSB medium over a 20 hour incubation period. It can be seen from the graph that cell number and cell density only slightly changed in the initial period (0-4 hours) and dramatically rose up after 4-6 hours of incubation. The broth pH has a slight change at the start and then greatly decreases after the 6th hour.

All three curves show behaviour consistent with the classical bacterial growth curve. From these data the 8th hour and the 13th hour of incubation of E. coli K12 cultured in TSB were chosen as representative of the mid stage of log phase and the early stage of stationary phase respectively.

L. acidophilus

The population growth curves of L. acidophilus are shown in Fig. 2, and the same classical behaviour is observed. However, different curves are seen depending on whether the samples are inoculated using a loop (replications 1-4) or using an inoculation of 1% by volume (replications 5-6). The higher initial cell numbers in the latter 2 samples causes the log phase and stationary phases to both be reached approximately 5 hours earlier.

From these results, it was decided to use the 1% by volume inoculation method for the spray drying experiments, and harvest at 6 and 10 hours for representative samples of the mid-log phase and the early stationary phase of L. acidophilus respectively.
Spray drying of E. coli K12

The survival rates of spray dried E. coli K12 (feed solution E1), from the mid-log phase and the early-stationary phase and at different drying outlet temperatures (60-100°C) are shown in Fig. 3. These clearly show cell survival decreasing with increasing temperature for bacteria from both growth phases, although 20% survival is still maintained even at 100°C outlet temperature.

The reasonable explanation for this temperature variation might be that cell wall of Gram negative bacteria as E. coli K12 is very thin and is broken easily at high temperature.

Bacteria from the early stationary phase show clearly better survival rates than those from the mid-log phase, indicating that the stage of growth affects the heat tolerance of bacteria, and this is
consistent with other studies (Corcoran et al., 2004). The best survival rates were found for bacteria in the early stationary phase with outlet temperatures of 60-80°C.

It is thus evident that the stage of growth has an influence on the heat tolerance of cells during spray drying. A possible explanation for this might be that in the stationary phase cell growth from the medium nutrients is limited, while subsequent secondary metabolism takes place, producing some toxins. Thus the cells have to adjust themselves to this harsher environment and generate a better ability to cope with external stresses (Meng et al., 2008; Corcoran et al., 2004; Morgan et al., 2006).

Spray drying of \textit{L. acidophilus}

Problems were encountered spray drying cell suspensions containing the MRS broth (Feed solution L1) due to product sticking on the walls of the chamber. Product was only obtained at two temperatures. Thus an evaluation of temperature dependence was made using the cell pastes/maltodextrin formulation (feed solution L2).

Fig. 4 shows the average survival rate of \textit{L. acidophilus} from the-mid log phase (the 6th hour of incubation) and the early stationary phase (the 10th hour of incubation) after spray drying feed solution L2 at drying outlet temperatures of 60, 70, 80, 90 and 100°C. As with \textit{E. coli K12} increasing temperature caused a reduction in cell survival and bacteria in the early stationary phase showing greater survival rates. This is in agreement with other studies on \textit{E. coli K12} (Van De Guchte et al., 2002; Teixeira et al., 1995a; Teixeira et al., 1995b). Unlike \textit{E. coli K12} the highest survival rates were found only at a temperature of 60°C for both growth phases (whereas \textit{E. coli K12} showed similar survival rates for the stationary phase bacteria at 60, 70 and 80°C).

\textbf{Direct comparison of survival rates between spray dried \textit{E. coli K12} and \textit{L. acidophilus} from cultured broth}

Fig. 5 shows the comparison of cell survival between spray dried \textit{E. coli K12} and \textit{L. acidophilus}, either in the mid- log phase or in the early-stationary phase after spray drying at outlet temperature of 70 and 80°C when spraying drying cultured broth (containing either TSB or MRS respectively) mixed with maltodextrin (Feed solutions E1 and L1). The results generally show that \textit{E. coli K12} has a lower survival rate than \textit{L. acidophilus} both in the mid-log phase and in the early-stationary phase at drying temperatures of 70 and 80°C.

It can be seen from Fig 5. that the survival rate of \textit{L. acidophilus} from the mid-log phase and the early-stationary phase after spray drying at 70°C is about 95 and 74%, whereas their survival at 80°C is about 67 and 66%, respectively. Comparing with the mid-log phase cell, \textit{L. acidophilus} has twice the cell survival than \textit{E. coli K12}. However, it is not clear whether this shows a genuine difference in cell survivability as there may be secondary effects of the drying medium on the drying behaviour of the droplets (Meng et al., 2008).
CONCLUSIONS

This study has investigated the heat tolerance of _E. coli K12_ and _L. acidophilus_ to the spray drying process. The study was designed to investigate the effect of growth phase and outlet drying temperature on cell survival rate.

The results of population growth study show that _E. coli K12_ took 8 hours to enter the mid-log phase and 13 hours to enter the early-stationary phase. While _L. acidophilus_ with 1% v/v inoculated volume entered the mid-log phase and the early-stationary phase at the 6th hour and the 10th hour of incubation time respectively. Data for cell density and pH of cultured broth confirmed that the cells entered either the mid-log phase or the early-stationary phase at the same period as shown by cell number measurements.

The spray drying study shows that outlet air temperature in spray drying has a significant effect on survival rate. At drying temperature of 60-80°C, survival rate of the mid-log phase _E. coli K12_ has been reduced about 6 Log10 CFU/mL, and has been dropped over 6 log10 CFU/mL at drying temperature of 90-100°C. Similar to cultured in early-stationary phase, the study presented that cell survival rate significantly decreased at drying temperature of 90-100°C around 2.5-5 Log10 CFU/mL.

Similarly, the rate of survival of _L. acidophilus_, either in the mid-log phase or in the early-stationary phase cell after spray drying (used cell paste mixed with 20% w/v maltodextrin as feed solution) suffers a large drop when spray drying at higher outlet temperatures. Also spray drying of the mid-log phase cells at outlet temperatures of 60°C has more than three times the survival rate when
spray drying at 100°C. Similarly, it was found that the survival rate of the early-stationary phase cell of *L. acidophilus* drops from 80% to 28% when changing drying temperatures from 60 to 80°C.

The relatively constant survival rates shown by early stationary phase *E. coli K12* between 60 and 80°C are interesting. It is well known that bacteria are most damaged under conditions of high temperature (Meng et al., 2008), while high moisture content affects the quality of spray dried products (Masters, 1991). Thus the existence of this plateau is presumably due to two competing effects. One is that increasing the outlet air temperature increases the temperature that the droplets are subjected to and thus reduces cell survival, but also that reducing the outlet air temperature increases the drying time (i.e. the time they are kept wet) and the time window during which cell inactivation can occur.

From the study, it can be concluded that cells harvested in the early-stationary phase had a greater heat resistance than those cultured in their mid-log phase at the same outlet temperature. When the two bacteria in the mid-log phase and in the early stationary phase were spray dried at 80°C with their own broth material they showed similar rates of cell survival. However, when using only 20% w/v maltodextrin as drying agent it was showed that *L. acidophilus* is stronger than *E. coli K12* (irrespective of the growth phase). This may be due to the much thicker cell wall of the Gram positive *L. acidophilus* bacteria.

REFERENCES


