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DIFFERENTIAL AIR OUTLET TEMPERATURES TO IMPROVE SURVIVAL OF ENDOPHYTIC BACTERIA DURING SPRAY DRYING

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ABSTRACT - *Endophytic bacteria Bacillus subtilis strain LKM-BL isolated within tissues of healthy Theobroma cacao L. plants was subjected to spray dry to develop powder-form product of biological control agent. In this study, we evaluated applicability of using spray dry technique with different air outlet temperatures of 75, 80 and 85°C on survivability and moisture content of product. The endophytic bacteria culture was formulated with 10% (w/v) skimmed milk as carrier and heated with constant air inlet temperature of 120 ± 2°C using B-290 mini spray dryer. The result revealed that 75°C air outlet temperature gave the highest survival rate of 62% with suitable moisture contents of 3.77%. Thus, the development of powder-form biological control agent endophytic bacteria B. subtilis LKM-BL via spray drying has been proven as a potential technique to improve cells viability prior heat adaptation.*

Key words*:* Endophytic bacteria, Spray drying, Temperature, Survivability, and Moisture contents

INTRODUCTION

Endophytic bacteria create a centre of attention because of the demand to bring down the use of toxic fungicide and fertilizer chemicals in agriculture, especially when concerned about environmental protection and quality of cocoa bean. The application of endophytes as bio-fungicide and bio-fertilizer in the powder-form, from a commercialization point of view, with an inexpensive method for large-scale production of powder cultures containing high levels of cells survival and suitable for their applications, is highly desirable. Drying and storage of bacterial cells is usually done using spray-drying technique for large-scale production (Gardiner et al. 2000).

This technique can be used to produce large amounts of endophytes powder-form product which are relatively inexpensive and stable for prolonged periods of storage. The drying process using a spray drying lower the production cost by 10 folds compared to the use of freeze-drying (Schuck et al. 2013). The resulting powdered products are highly suitable for application, easy to transport at low cost, can be stored in stable form for a long time (Gardiner *et al.,* 2000) and can be easily applied at cocoa plantations.

However the spray dry technique can damage the cells through heat shock and abrupt removal of water from cells, leading to cell shrinkage and desiccation. Thus, multi-step of optimization of parameters is crucial to produce high level of cells survivability. The main objectives of the study are to determine effectiveness of different spray drying air outlet temperatures against percentage of survivability and relative humidity of powder-form product. The study will be conducted using endophytic bacteria *Bacillus subtilis* strain LKM-BL that was previously isolated from internal tissue of healthy cocoa plant. The strain is capable of producing antifungal substances against cocoa pathogens ad as well as producing plant growth hormones.

MATERIALS AND METHODS

Endophytic Bacterial.

The culture of endophytic bacteria *Bacillus subtilis strain LKM-BL* was obtained from the Microbiology Laboratory of Biotechnology Division, Malaysian Cocoa Board. Stock culture of each isolate was maintained at -80ºC in nutrient broth with 15% glycerol (Shin *et al*., 2007). The working cultures were established by transferring from stock cultures onto nutrient agar (NA) in Petri dishes and incubated for 24 h at 28ºC.

Preparation of Culture.

Endophytic bacteria *B. subtilis* LKM-BL culture was transferred from stock culture onto fresh nutrient agar (NA) and incubated for 24 hours at 28ºC. After 24 hours, single colony was transferred to nutrient broth (NB) and incubated in the same condition, stirring at 250 rpm. This broth was then used to inoculate a second NB (1% *v*/*v*) to reach stationary phase. Bacteria cell pellets were harvested by centrifugation at 10 000 *g* for 10 min at 4ºC. Then the harvested cell pellets were mixed into 1000 ml of 10% (w/v) sterile skim milk as carrier which gave an initial number of approximately 10^9 CFU/ml.

Spray-Drying at Different Air Outlet Temperature.

The sample was spray-dried at constant air inlet temperature of 120 ± 2ºC using B-290 mini spray dryer. The flow rate of the feed was varied to obtain air outlet temperature of 75 ± 2ºC, 80 ± 2ºC and 85 ± 2ºC. Spray dried powders were collected and stored in tight sealed container. The experiments were repeated at least three times. Viable counts were plated on NA and were converted to log CFU/ml and percentages were calculated.

Observation of Spray-Drying Powder-form.

Visual observation was investigated by the appearance and the particle colour of spray-drying powdered.

Determination of Bacteria Cells Survivability in Spray-Drying Powder-form.

The percentage of bacterial cell survivability in spray-drying was evaluated based on;

$$\textit{Survivability \%} = (N / No) \times 100$$

Where N is the number of bacteria after drying and No is the number of bacterial cells before drying (Radulovic et al. 2012). The number of endophytic bacterial cells was measured by estimating the number of the colony forming units (CFU/g) of cultures was assessed by plate count method.

Determination of Moisture Content in Spray-Drying powder-form.

The moisture content of spray-drying powder was determined by oven drying at 102ºC for 24 hours. This involved determination of the difference in weight before and after drying, expressed as a percentage of the initial powder weight, according to International Dairy Federation Bulletin (IDF 1993) (Corcoron et al. 2004).

Statistical Analysis

All data results obtained were analyzed using SPSS versión 13.0 Windows program (SPSS Inc., Chicago, IL) using one-way variance analysis (ANOVA). Duncan tests were performed to find out the significant differences for each data obtained at $p < 0.05$.

RESULTS AND DISCUSSIONS

After the spray drying using different air outlet temperatures of 75 ± 2ºC, 80 ± 2ºC and 85 ± 2ºC found that, the physical properties appearance of powder particles were varied. Observation found that the powdered product of *B. subtilis* LKM-BL appeared to be white and bright at a drying temperature of 75 ± 2ºC. The higher of drying air outlet temperature of the spray drying at 80 ± 2ºC and 85 ± 2ºC seems likely the particles of the powder product became browning and clumping (Fig. 1.0). This is due to high air outlet temperature present during drying activity.

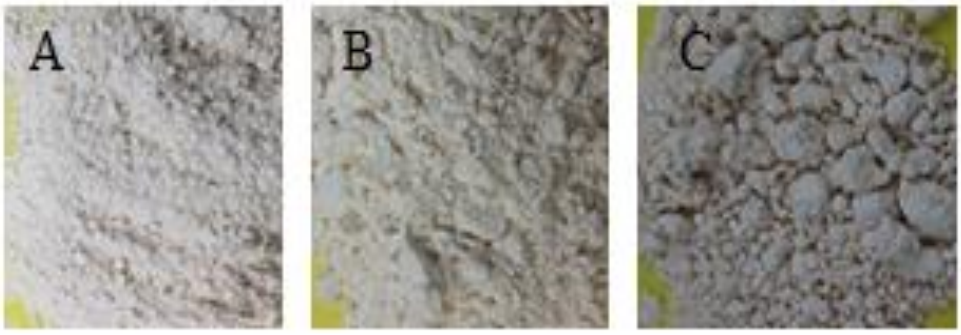

Figure 1.0 Physical properties of powdered particles at (A) 75±2ºC, (B) 80±2ºC and (C) 85±2ºC air outlet temperature of spray dry.

The results revealed that 75 ± 2ºC air outlet temperature gave the highest survival rate of 62% bacteria cells compared to air outlet temperature of 80 ± 2ºC of 54% cells and 85 ± 2ºC with survival rate of 44% (Fig. 2.0). The survival of bacteria cells decreased with increasing of air outlet temperature due to thermal degradation of bacteria cells. Statistical analysis showed significant differences ($p<0.05$) were obtained from the comparative results of percentage of survivability bacteria cells against different air outlet temperatures.

According to Nicholson et al. (2000), the genus *Bacillus*, especially the endosporous *B. subtilis*, is resistant to high temperatures of drying. Cell bacteria become dormant for a long time when the bacterial cells form a powder. The results from this experiment showed that *B. subtilis* LKM-BL was able to survive at 75 to 85ºC when undergoing spray drying to produce a powder-form product. The spray drying process has also been reported on the bacterial biological control agent of *B. thuringiensis*. According to Adjalle et al. (2011) spray-drying method is an economical process and performed a stable powder-form product compared to the freeze-drying. Other similar study conducted by Yanez-Mendizabal et al. (2012) showed that *B. subtilis* CPA-8 was resistant up to 80ºC temperature of spray-drying. The powder-form product was used as anti-fungal control agents of *Monilinia spp*. which attacks the roots of peach plants. The percentage of bacterial survival of *B. subtilis* CPA-8 after spray drying was 28-32% using 10% skim milk + 10% magnesium sulphate (MgSO4).

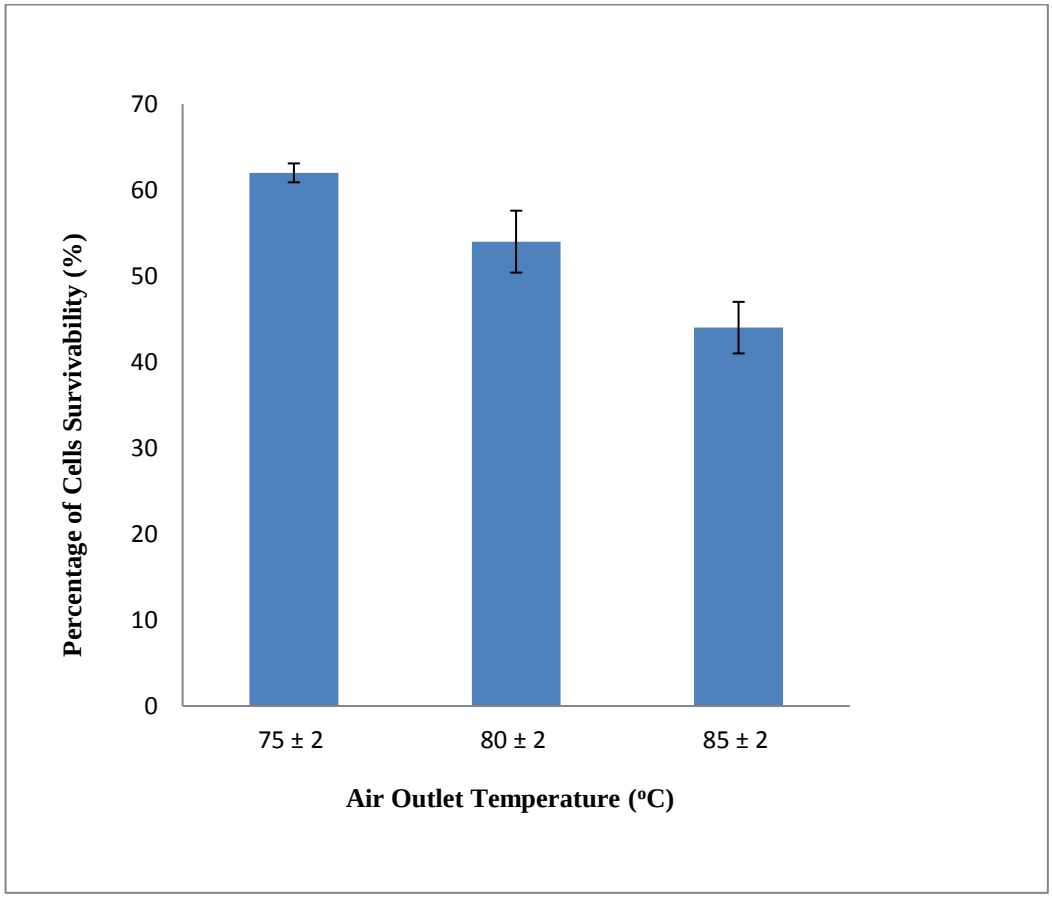

Figure 2.0 Effect of spray-drying endophytic bacteria B. subtilis LKM-BL powdered using different air outlet temperatures against percentage of cells survivability.

Endophytic bacterial LKM-BL spray-drying powder-form was also determined moisture content using oven drying. The powder-form product was dried at 102ºC for 24 hours. The results showed that the percentage of moisture content of *B. subtilis* LKM-BL powder-form by spray-drying at air outlet temperature of 75 ± 2ºC, 80 ± 2ºC and 85 ± 2ºC decreased as the temperature increased (Fig. 3.0). At the drying air outlet temperature of 75±2ºC, the result showed the highest moisture content of 3.77 ± 0.2%. While the air outlet temperature of 80 ± 2ºC gave a decrease in moisture content of 2.73 ± 0.1% followed by an air outlet temperature of 85 ± 2ºC resulted in the lowest moisture content of 2.43 ± 0.1%. High air outlet temperatures resulted in *B. subtilis* LKM-BL powder-form product losing humidity. Statistical analysis showed that the spray-drying air outlet temperature of 75 ± 2ºC produced significantly the highest percentage of moisture content ($p<0.05$) compared to 80 ± 2ºC and 85 ± 2ºC. Meanwhile, each temperature 80 ± 2ºC and 85±2ºC showed significantly ($p<0.05$) difference in moisture content respectively, between them.

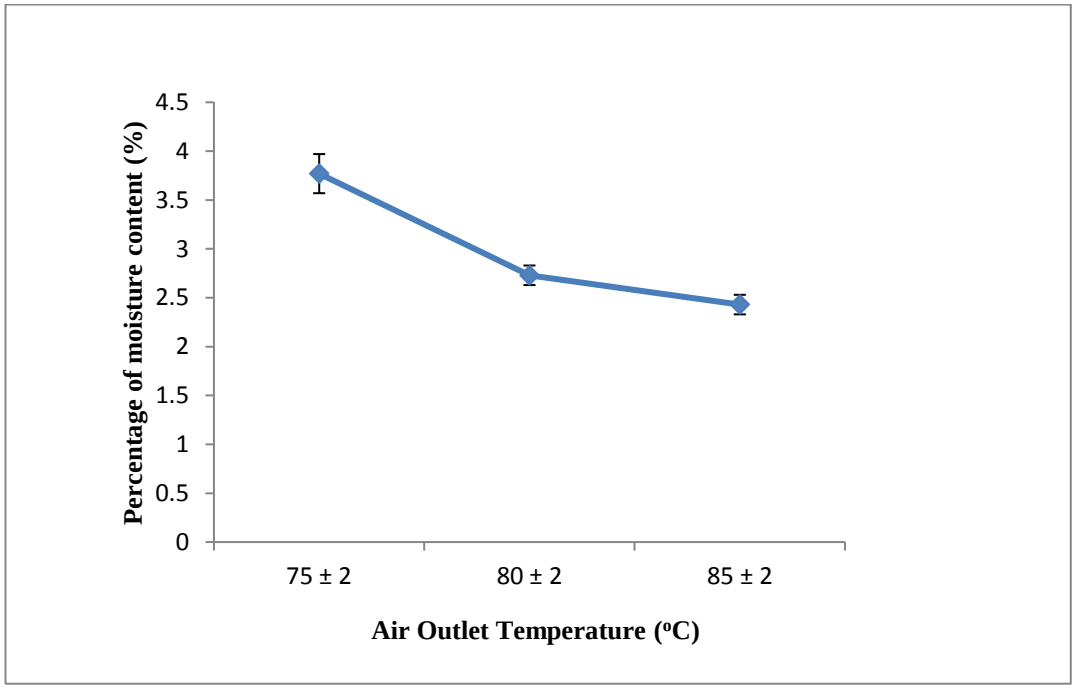

Figure 3.0 Effect of spray-drying endophytic bacteria B. subtilis LKM-BL powdered using different air outlet temperatures against moisture content.

The results showed that *B. subtilis* LKM-BL powder-form moisture content at different air outlet temperature revealed humidity below 4% with high survivability of the bacterial cells. Moisture content affects spray-drying powder-form products and the appropriate percentage of moisture content is about 4% (Master 1985). Similar study was performed against *Lactobacillus paracasei* SD1 to produce powder-form products but exceeded above 4% standard humidity of 6 to 6.89% when dry spray temperature was maintained at 60 to 70°C. Dry spray temperatures were increased from 80 to 90°C to achieve a humidity percentage below 4% (Teanpaisan et al. 2012). Similar results also were obtained conducted on *L. acidophilus* and *L. salivarius* where high temperatures were required at 80 to 85°C so that the moisture content did not exceed 4% but unfortunately resulted low survivability of cells (Gardiner et al. 2000).

CONCLUSIONS

Findings showed that the visual observation on physical properties appearance of spray-drying powder-form particles, varied at different air outlet temperature of spray dry. The survival rate of bacteria cells decreased with increasing of air outlet temperature. Production of endophytic bacteria powder *B. subtilis* LKM-BL in 10% skim milk at 75 ± 2°C was the most suitable between the three air outlet temperatures studied with moisture content of 3.77% and the number of cell survivability in the powder-form product of about 62%. Overall, the results of this study found that the endophytic bacterium *B. subtilis* LKM-BL could be performed into powder-form product with specific spray-drying air outlet parameters and used as a natural biological control agent.

ACKNOLEDGEMENT

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