UNIVERSITI TEKNOLOGI MARA

SPRAY DRIED FORMULATION OF *Bacillus subtilis* UiTMB1 TO CONTROL OF BACTERIAL LEAF BLIGHT (BLB) CAUSED BY *Xanthomonas oryzae* pv. *oryzae*

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This dissertation has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

The present study focuses on development of sustainable and reliable formulation of *Bacillus subtilis* UiTMB1 to control *Xoo* with low-cost substrates such as molasses and soybean curd residue (SCR) using a spray drying technique. The molasses and bio-waste substrate SCR were selected as carbon and nitrogen sources for the bacterial strain. In this study, 10 different concentrations of molasses from 1% to 10% [w/v]) were suspended in distilled water containing 10^6 CFU mL^{-1} of *B. subtilis*UiTMB1 and incubated for 24 hours. Out of 10 tested concentrations, 5% molasses that gave the highest bacterial cell production was selected for a subsequent experiment with SCR powder in a basal salt solution. Three concentrations of SCR powders which0.5, 1.5 and 3.0% (w/v) were used to evaluate the optimal concentration of SCR for the production of whole cells and endospore cells as well as their spore efficiency (%). In the results, the formulation of 5% molasses combined with 1.5% SCR in basal salt solution has yielded 1.77×10^8 CFU mL^{-1}whole-cells and 2.47×10^8 CFU $mL^{-1}$of endospores. The formulation also gave the highest percentage of spore production at 92.7%. The effects of survivability (%) and the viability of spray-dried *B. subtilis* UiTMB1 were further evaluated upon incorporation with three different carriers of 10% magnesium sulphate ($MgSO_4$), 7.5% $MgSO_4$ + 2.5% SCR, and 5% $MgSO_4$ + 5% SCR for spray drying produced by the spray dryer with inlet temperature of 150 ± 2°C and outlet temperature of 80± 2°C. Six months of storage at two different temperatures which were 4±2°C and 24±2°C for *B. subtilis* UiTMB1 formulation incorporated with carrier were evaluated for their viability during the storage. The finding showed that all carriers promoted 95.7–93.2% of survivability of *B. subtilis* UiTMB1 after the spray drying process. However, significant reductions of bacterial colonies in all spray dried *B. subtilis* formulations can be observed over the six months storage duration before constant at 10^7 CFU mL^{-1}.The spray dried *B. subtilis* UiTMB1 formulations were further evaluated using agar disc diffusion method and rain shelter study to test their efficacy against BLB pathogen. The *in-vitro* study showed that all formulations were able to inhibit the growth of pathogen with formation of clear zones. The average diameter (mm) of clear zones for spray dried *B. subtilis*UiTMB1 formulations between 14.10-14.60 mm were significantly higher in inhibition of *Xoo* growth on Muller-Hilton agar plates compared to the positive controls plates. Meanwhile, *in-vivo* study recorded that all formulations were able to reduce the BLB disease between 46.4 – 53.4% with significantly low of infection rate at 0.025-0.030 unit. Among the three formulations, the spray dried of *B. subtilis* UiTMB1 with 10% $MgSO_4$ (T1) offered the highest disease reduction (53.4%) and lowest area under disease progress curve (AUDPC), (256.4 $unit^2$). This formulation has a huge potential to be further explored as reliable biological product for BLB management with low cost production and acceptable shelf life.

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