UNIVERSITI TEKNOLOGI MARA

OPTIMIZATION OF Aloe Vera Barbadensis Miller MEDIUM FOR Lactobacillus plantarum NBRC 3070 CULTIVATION USING RESPONSE SURFACE METHODOLOGY (RSM)

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Thesis submitted in fulfillment of the requirements for the degree of **Master of Science**

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 13th January 2016 to conduct the final examination of Hifa Nazirah binti Mohammed Yaziz on his Master of Science thesis entitled "Optimization of *Aloe Vera Barbadensis Miller* Medium for *Lactobacillus plantarum* NBRC 3070 Cultivation using Response Surface Methodology (RSM)" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners was as follows:

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis 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

Aloe vera has been hypothesized as an alternative prebiotic source for probiotic cultivation due to high content of carbohydrate. However, to date the use of *Aloe vera* as prebiotic has not been reported elsewhere. The aim of this study was to optimize Aloe vera medium supplemented with various carbon and nitrogen components to obtain the optimum Lactobacillus plantarum NBRC 3070 cell biomass production. Fractional Factorial Design (FFD) and steepest ascent were employed to identify the significant factors among the medium components on the cell growth and to approach proximity of optimum. Based on the screening step, maximum biomass production obtained was 11.816 log₁₀ CFU/ mL. As for regression analysis output, high Aloe vera compositions with glucose supplement were found to be influenced biomass production significantly (P < 0.05). Consequently, both factors were chosen for further step, steepest ascent. Result from the steepest ascent revealed that biomass production was increased with the increased of Aloe vera and glucose composition until up to 3.8% (v/v) and 5.5% (w/v), respectively. However, further increased in both components resulted in cell reduction. The highest L. plantarum NBRC 3070 biomass production was recorded at the 6th steepest ascent path with 10.105 log₁₀ CFU/ mL. Consequently, Aloe vera with 3.8% (v/v) and glucose with 5.5% (w/v) were used as a middle point in further optimization process. The optimum values were determined by Central Composite Design (CCD) under Response Surface Methodology (RSM) to optimize the use of Aloe vera and glucose. The statistical analysis showed that the optimal compositions of Aloe vera gel and glucose were at 3.65% (v/v) and 5.52% (w/v), respectively by setting the goal of optimization for biomass production to be at maximum level and the factor concentration in selected range. Predicted response in optimal conditions (9.98 log₁₀ CFU/ mL) was generated by the statistical tool. Based on the verification process, the experimental and predicted results were not significant difference (P>0.05) with low error percentage. The optimized Aloe vera and glucose medium allowed a highest cell biomass production up to 9.86 log₁₀ CFU/ mL which 18% higher than in de Man Rogosa Sharpe (MRS) cultivation medium. The optimized Aloe vera-glucose medium was further tested with other probiotic strains (Lactobacillus casei ATCC 393, Lactobacillus reuteri ATCC 55730, Lactobacillus acidophilus ATCC 4356 and Bifidobacterium pseudocatenulatum ATCC 27919) and it has been proved that this optimized medium was able to support their growth. Based on carbohydrate profiling, both Aloe vera gel and glucose were utilized by L. plantarum NBRC 3070 throughout cultivation process as carbon source for growth. Meanwhile, pH profiling showed the reduction of pH value occurred during cultivation due to organic acid produced from carbohydrate metabolism.

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