UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF MEDIUM FORMULATION FOR FOLATE BIOSYNTHESIS BY *Lactobacillus plantarum* I-UL4 USING RESPONSE SURFACE METHODOLOGY

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FBSB 2011 33

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master Science

May 2011

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in the fulfillment of the requirement of the Degree of Master Science

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Chairman : Assoc. Prof. Rosfarizan Mohamad, PhD

Faculty : Biotechnology and Biomolecular Sciences

Folate plays an important role in human life as one of the most important components for the synthesis of purine, guanine, adenine, pyrimidine and thymine. In this study, lactic acid bacteria (*Lactococcus lactis* NZ 9000 and MG 1363, *Lactobacillus plantarum* I-UL4, and *Lactobacillus johnsonii* DSM 20553) were investigated for the biosynthesis of folate. The total folate concentrations in lactic acid bacteria were determined by microbiological assay as a reliable method to evaluate the folate level and *Lactobacillus rhamnosus* (*Lactobacillus casei*, ATCC 7469) was used to analyze total folate and responds to most native folates. From this study, all lactic acid bacteria able to synthesized folate and *Lb. plantarum* I-UL4 exhibited as the best folate biosynthesis and used throughout investigation on the medium optimization.

Five components of medium composition (carbon source, lactose, nitrogen source, meat extract and *p*-aminobenzoic acid (PABA)) were optimized conventionally and the

cultivation process was conducted in shake-flask experiment. From the investigation, lactose, meat extract and PABA at concentration 20 g/L, 15 g/L and 10 µM respectively gave significant effect towards growth of *Lb. plantarum* I-UL4 and folate biosynthesis. Thus, these three concentrations were subsequently used for further medium optimization study based on response surface methodology (RSM).

Through RSM approach, twenty experiments of three factors in response to folate biosynthesis were carried out. Estimated optimize conditions of the factors for the growth of *Lb. plantarum* I-UL4 and folate biosynthesis as suggested by RSM are as follows: lactose, 20 g/L; meat extract, 16.57 g/L and PABA concentration, 10 µM. The optimized medium composition was then further applied in the cultivation process using 2 L stirred tank bioreactor. Finally, about 61.89 µg/L of folate was synthesized by *Lb. plantarum* I-UL4 using the bioreactor, which was slightly higher than in shake-flask experiment (59.88 µg/L) using the optimized medium composition. In a conclusion, a better understanding of the relationship among the factors was obtained by RSM, which was used as a statistical tool to improve the folate biosynthesis of *Lb. plantarum* I-UL4 in small and large scale cultivation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN FORMULASI MEDIA UNTUK BIOSINTESIS FOLAT OLEH *Lactobacillus plantarum* I-UL4 MENGGUNAKAN KAEDAH RANGSANGAN PERMUKAAN

Oleh

NORFARINA BT MUHAMAD NOR

Mei 2011

Pengerusi : Profesor Madya Rosfarizan Mohamad, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Folat memainkan peranan yang penting dalam kehidupan manusia dimana ianya digunakan sebagai satu komponen untuk sintesis purin, guanin, adenin, piarimidin dan tiamin. Dalam kajian ini, bakteria asid laktik (*Lactococcus lactis* NZ 9000 and MG 1363, *Lactobacillus plantarum* I-UL4, dan *Lactobacillus johnsonii* DSM 20553) telah di uji untuk biosintesis folat. Jumlah kepekatan folat dalam bakteria asid laktik ditentukan dengan kaedah mikrobiologi iaitu kaedah yang tepat untuk menilai tahap folat dan *Lactobacillus rhamnosus* (*Lactobacillus casei*, ATCC 7469) digunakan untuk menganalisis jumlah folat dan semua folat natif. Dalam kajian ini, semua bakteria asid laktik boleh mensintesiskan folat dan *Lb. plantarum* I-UL4 telah menunjukkan keupayaan untuk biosintesis folat yang terbaik dan digunakan dalam seluruh kajian pengoptimuman medium.

Lima komponen komposisi media (sumber karbon, laktosa, sumber nitrogen, ekstrak daging dan asid p-aminobenzoik) telah di optimumkan secara konvensional dan proses pertumbuhan ini dijalankan di dalam kelalang kon. Daripada kajian, laktosa, ekstrak daging dan PABA pada kepekatan 20 g/L, 15 g/L dan 10 µM masing-masing memberikan kesan pada pertumbuhan *Lb. plantarum* I-UL4 dan folat biosintesisnya. Demikian juga tiga kepekatan ini digunakan untuk kajian seterusnya dalam pengoptimuman medium berasaskan kaedah rangsangan permukaan (RSM).

Melalui kaedah rangsangan permukaan, dua puluh eksperimen untuk tiga faktor telah dijalankan. Anggaran kondisi faktor yang optimum untuk pertumbuhan *Lb. plantarum* I-UL4 dan penghasilan folat seperti yang disyorkan oleh RSM adalah seperti berikut: laktosa, 20 g/L; ekstrak daging, 16.57 g/L dan kepekatan PABA, 10 µM masing-masing. Medium yang optimum pada proses pertumbuhan seterusnya telah dikaji menggunakan bioreaktor 2 L yang berpengaduk. Akhir sekali, biosintesis folat oleh *Lb. plantarum* I-UL4 menghasilkan folat sebanyak 61.89 µg/L. Dengan menggunakan medium yang optimum yang sama seperti di dalam bioreaktor, folat di dalam kelalang kon dihasilkan sebanyak 59.88 µg/L. Sebagai kesimpulan, kefahaman yang mendalam mengenai hubungan antara faktor-faktor tersebut telah diperolehi dari RSM, dimana ianya digunakan sebagai alat statistik untuk memperbaiki biosintesis folat oleh *Lb. plantarum* I-UL4 dalam skala yang kecil mahupun yang besar.

ACKNOWLEDGEMENT

All praise to ALLAH S.W.T who has guide my safety, give me a great health that I could not adequately thank for. The strength given by ALLAH has provided me to finish my study. Alhamdulillah.

I would like to express a great gratitude to my supervisor, Assoc. Prof. Dr. Rosfarizan Mohamad, who have always had enormous patience and helped me in every way possible. Thanks for the great help. My gratitude also goes to my co-supervisors, Prof Dr. Raha Abdul Rahim and Assoc. Prof. Dr. Foo Hooi Ling for their invaluable guidance, constant encouragement and constructive suggestions throughout this study. Thanks also extended to my amazing friends at the Department Bioprocess Technology, who are directly or indirectly supporting and helping.

I could not finish these lines without extending special thanks to my husband, Khairil Amri b. Anuwar for his continuous support and encouragement during the realization of this work. He really deserves all the love and trust that I have placed in him. To my baby Nurfaliha Qaisara, thanks for your smile. Your smiles always brighten my day life. Finally, I would like to mention the invaluable help, enormous support, endless loves, cares and patience to my family members. My higher gratitude also expressed to my beloved father, Mr. Muhamad Nor bin Sahar and my beloved mother, Madam Amanah bt Sajak for really understand with my study. All prayers from both of you have brought success to my life. Thank you for all.

I certify that an Examination Committee has met on 24/05/2011 to conduct the final examination of Norfarina bt Muhamad Nor on her thesis entitled "Optimization Of Medium Formulation for Folate Biosynthesis by *Lactobacillus Plantarum* I-UL4 Using Response Surface Methodology" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science Industrial Biotechnology.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citation, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other at Universiti Putra Malaysia or other institutions.

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NORFARINA BT MUHAMAD NOR

Date: 24th May 2011

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