UNIVERSITI PUTRA MALAYSIA

CELL VIABILITY OF BIFIDOBACTERIUM UNDER FREEZE DRYING, STORAGE AND GASTROINTESTINAL TRACT SIMULATION BY MICROENCAPSULATION

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FBSB 2011 5

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By

SEYEDEH FATEMEH SHAMEKHI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

March 2011

Dedicated to:

My son, all of my hope and motivation for the life
&
My beloved parents

Abstract of Thesis presented to the Senate of Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of Science

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Chairman: Associate Professor Shuhaimi bin Mustafa, PhD

Faculty: Biotechnology and Biomolecular Sciences

Nowadays, it is well known that many infants in the world who are deprived of breast feeding and in the face of high incidence of multiple gut related diseases, need to be supplied with a formula capable of substituting breast milk's synbiotic properties. The purpose of this research was to improve the viability of probiotic bacteria (*Bifidobacterium lactis* DSM 10140 and *Bifidobacterium infantis* DSM 20088) during freeze-drying process, storage period and infantile gastrointestinal tract (GIT) conditions by microencapsulation using prebiotics. Special emphasis was given to the use of pediatrics recommended prebiotics mixture including galactooligosaccharides (GOS) and fructooligosaccharides (FOS). Initially, probiotic microorganisms were encapsulated with a coat combination of prebiotics-calcium-alginate prior to freeze-drying, using emulsion technique. Glycerol was

used as cryo-protectant to enhance the survival of beads over freeze-drying. Both encapsulated and free cells were then freeze dried in their optimized combinations of skim milk and prebiotics as freezing media. Statistical optimization techniques were used to produce a coating combination as well as drying medium for each strain with the highest survival during freeze-drying. The interactive effects of Na-alginate, prebiotics and glycerol as well as skim milk and prebiotics on the viability of encapsulated and free cells were determined respectively. The statistical optimizations were performed based on Response Surface Methodology (RSM). The inputs, percentage survival, were derived experimentally and tested by RSM. The optimum compositions for encapsulation of *B. lactis* and *B. infantis* derived via RSM analysis were: Na-alginate 2.1% and 2.9%, prebiotic 2.9% and 2.7% and glycerol 21.7% and 25.4%, respectively. Maximum survival of encapsulated *B. lactis* and *B. infantis*, predicted by models during freeze drying were 81.2% and 72.1%, whereas those of free cells (as control) were 62.1% and 47.6%, respectively. No significant ($p > 0.05$) difference between the predicted and experimental values verified the adequacy of all final reduced models fitted by RSM. The protective effects of encapsulation on survival rates of cells as compared to free cells were evaluated over a storage period. After 120 days of storage of encapsulated cells at 4˚C, there was about 1 $\log_{10}$ cfu/ml improvement in the viability of both strains as compared to free cells. Also, two different simulated infantile GIT conditions including gastric conditions (pH 3.0 and 4.0, 90 min, 37ºC) and intestinal conditions (pH 7.5, 5h, 37ºC) in a sequential model were conducted for assessment of both free and encapsulated cells' survival. The mortality rates of encapsulated *B. lactis* 10140 after sequential incubation in simulated GIT conditions, when it had passed the gastric juice at pH 3.0 and 4.0 were reduced by 1.28 and 0.5 $\log_{10}$ cfu/ml, as compared to those of free

cells. For *B. infantis* 20099, these reduction rates were 2.14 and 1.47, respectively. From this study, it can be concluded that microencapsulation of *B. lactis* 10140 and *B. infantis* 20088 using prebiotics, was a successful effort to produce a stable synbiotic powdery nutraceutical.

Abstrak Tesis Untuk Dikemukakan Kepada Senat Universiti Putra Malaysia Sebagai Memenuhi Keperluan Untuk ijazah Master Sains

KEMANDIRIAN SEL *BIFIDOBACTERIUM* SEMASA PENGERINGAN SEJUK BEKU, PENYIMPANAN DAN SIMULASI SALURAN PENGHADAMAN MELALUI MIKROENKAPSULASI

Oleh

SEYEDEH FATEMEH SHAMEKHI

Mac 2011

Pengerusi : Profesor madya Shuhaimi bin Mustafa, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Pada masa kini, telah diketahui bahawa ramai bayi didunia kekurangan penyusuan susu ibu dan mengalami penyakit yang berkaitan dengan usus pada kekerapan yang tinggi. Oleh itu, mereka perlu diberi susu formula yang mampu menandingi susu ibu dari segi cirri prebiotiknya. Tujuan penyelidikan ini ialah untuk meningkatkan kehidupan bakteria probiotik (*Bifidobacterium lactis* DSM 10140 dan *Bifidobacterium infantis* DSM 20088) semasa pengeringan sejuk beku, penyimpanan dan pendedahan kepada keadaan didalam saluran penghadaman melalui pengkapsulan mikro menggunakan prebiotik. Penekanan khusus telah dberikan kepada saranan oleh pakar kanak-kanak iaitu campuran prebiotik termasuk galaktooligosakarida (GOS) dan fruktooligosakarida (FOS). Permulaannya, mikroorganisma probiotik dikapsulkan menggunakan kombinasi prebiotik-kalsium-alginat

sebelum proses pengeringan sejuk beku menggunakan teknik pengemulsian. Gliserol telah digunakan sebagai pelindung beku untuk meningkatkan kehidupan mikroorganisma semasa penyejukbekuan. Kedua-dua sel bebas dan sel yang dikapsulkan kemudian disejuk bekukan didalam kombinasi optimum susu skim dan prebiotik sebagai media penyejukan. Teknik pengoptimuman secara statistik telah digunakan untuk menghasilkan kombinasi penyalutan dan juga medium pengeringan untuk setiap strain dengan kehidupan tertinggi semasa pengeringan sejukbeku. Kesan interaktif antara Na-alginat, prebiotik dan gliserol serta susu skim dan prebiotik keatas kehidupan sel yang dikapsulkan dan sel bebas juga ditentukan. Pengoptimuman secara statistik telah dilaksanakan berasaskan kepada Metodologi Tindakbalas Permukaan (MTP). Input dan peratus kehidupan telah diperolehi secara eksperimen dan diuji menggunakan MTP. Komposisi yang optimum yang diperolehi untuk pengkapsulan *B. lactis* 10140 dan *B. infantis* 20088 melalui analisis MTP ialah: Na-alginat 2.1% dan 2.9%, prebiotik 2.9% dan 2.7% dan gliserol 21.7% dan 25.4%, masing-masing. Kehidupan maksimum yang diramalkan oleh model MTP untuk *B. lactis* 10140 dan *B. infantis* 20088 yang dikapsulkan semasa pengeringan sejukbeku ialah 81.22% dan 72.18% manakala sel bebasnya pula ialah 62.16% dan 47.63%, masing-masing. Tiada perbezaan ketara ($p > 0.05$) diantara nilai yang diramalkan dengan nilai eksperimen mengesahkan bahawa model yang dijana melalui MTP adalah mencukupi. Kesan perlindungan proses pengkapsulan keatas kehidupan bakteria yang diuji berbanding dengan sel bebas juga dinilai sepanjang tempoh penyimpanan. Selepas 120 hari penyimpanan sel yang dikapsulkan pada 4^{o}C, didapati sebanyak 1 $\log_{10}$ cfu/ml peningkatan kehidupan kedua –dua sel bakteria berbanding dengan sel yang tidak dikapsulkan. Juga, dua keadaan berbeza didalam saluran penghadaman telah disimulasikan iaitu keadaan didalam perut (pH 3.0 dan 4.0, 90 min,

37^{o}C) dan didalam usus (pH 7.5, 5 jam, 37^{o}C) secara berterusan telah dijalankan untuk menilai kehidupan kedua-dua sel yang dikapusalkan dan sel bebas. Kadar kematian *B. lactis* 10140 yang dikapsulkan semasa pendedahan secara berterusan didalam salauran penghadaman yang simulasikan iaitu apabila melalui jus gastrik pada pH 3.0 dan 4.0 telah menurun kepada 1.28 dan 0.36 log10 cfu/ml jika dibandingkan dengan sel yang tidak dikapsulkan. Untuk *B. infantis* 20088 pula, kadar penurunan ialah sebanyak 2.13 dan 1.47, masing-masing. Daripada kajian ini, dapat disimpulkan bahawa pengkapsulan mikro *B. lactis* 10140 dan *B. infantis* 20088 menggunakan prebiotik merupakan satu usaha yang berjaya untuk menghasilkan serbuk sinbiotik nutrasutikal yang stabil.

ACKNOWLEDGEMENTS

*In The Name of **ALLAH**, The Most Merciful and Most Beneficent*

First and foremost, a word of thanks is given to **ALLAH,** the source of all Knowledge, by whose abundant grace this work came to fruition.

I would like to express my sincerest appreciation to my supervisor **Associate. Prof. Dr. Shuhaimi Bin Mustafa**, for the opportunity to do this work. Fairly speaking, this work has been done from A – Z according to his ideas, kind guidance and invaluable advises and criticism throughout the work and particularly during writing this thesis. Special thanks go to the members of my supervisory committee **Professor Dr. Arbakariya Ariff** and **Professor Dr. Mohd Yazid Manap**. Their confidence, guidance, support, and catching optimism during my master studies were very important and continuously encouraging.

I would also like to thank to **Dr. Naji** for his valuable helps and to all of my friends at the Department of Bioprocess Technology, FBSB, UPM, especially to **Sahar** for their kindly guidance and supports.

Last but not least, I am immensely and forever grateful to my beloved parents, husband and sister for their love, supports, unending encouragement, highly appreciated patience day and night all over the time of my study and prays throughout my life. Allah bless them all forever.

I certify that an Examination Committee has met on March 21, 2011 to conduct the final examination of Seyedeh Fatemeh Shamekhi on her master thesis entitled " Improvement of *Bifidobacterium* Survival During Freeze Drying, Storage And Gastrointestinal Tract Transit By Encapsulation For Incorporation Into Infant Formulae" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of master of science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

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

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SEYEDEH FATEMEH SHAMEKHI

Date: 21 march 2011