UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF ALPHA-INTERFERON IN LACTOCOCCUS LACTIS

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PRODUCTION OF ALPHA-INTERFERON IN *LACTOCOCCUS LACTIS*

By

OMID BAYAT

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

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DEDICATION

Dedicated to my beloved mother, father, family, friends and well wishes for their love, interest and encouragement

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: Professor Raha binti Abdul Rahim, PhD

Faculty: Biotechnology and Biomolecular Sciences

Human interferon- 2b (IFN- 2b) is one of the members of IFN family and it is also one of the biopharmaceuticals used to cure diseases such as hairy cell leukemia, malignant melanoma, and chronic hepatitis (B and C). The majority of commercial products available in the market are obtained in ***E. coli***. The high production of ***E. coli*** is in the form of inclusion body (IB) which has to pass through costly refolding steps and also complicated purification procedures for removing of lipopolysaccharide (endotoxin) are required. In contrast, recombinant IFN- produced by ***L. lactis*** strain with GRAS status does not form IB and can be delivered at the mucosal level and does not require costly refolding steps and purification procedures. It can also be used as an adjuvant in designing new vaccines against viral infections. In this study, four recombinant ***L. lactis***, MGIF (containing P32, a constitutive promoter), PNZ, PNHIF, PNZUSPIF (containing Pnis, an inducible promoter)

were constructed for the expression of IFN-alph 2b. The production of IFN-2b was confirmed by ELISA and western blotting and the plasmid stability test showed that the recombinant plasmids were stable in the strains even after 100 generations. The effect of different carbon sources (glucose, sucrose and lactose), nisin induction and incubation time in M17 medium on the amount of production were also tested. The results showed that the highest production was achieved in presence of glucose for all the recombinant strains and the best concentration of nisin induction for recombinant strains with Pnis promoter was at 30 ng/mL. In addition, the highest expression amount of IFN for MG1363 recombinant (MGIF) was at 9 hours of incubation and for NZ9000 recombinants (NZIF and NZUSPIF) was at 4.5 hours. Among the four recombinant strains, the highest production amount with the optimum conditions was achieved by recombinant NZ9000 harbouring SP_{usp45}- IFN- 2b gene (~0.27 g/L). The expressed IFNs were subjected to bioactivity test and they showed acceptable bioactivity of 1.9×10^6 IU/mg. In conclusion, the results of this study proved that IFN-alpha 2b can be expressed in ***L. lactis*** with an acceptable level of bioactivity.

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

PENGHASILAN ALFA-INTERFERON DALAM *LACTOCOCCUS LACTIS*

Oleh

OMID BAYAT

Februari 2013

Pengerusi: Professor Raha binti Abdul Rahim, PhD

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Interferon- 2b (IFN- 2b) manusia tergolong dalam kumpulan interferon dan ia adalah salah satu bio-farmaseutikal yang digunakan untuk merawat penyakit seperti leukemia sel berbulu, melanoma malignan, dan hepatitis kronik (B dan C). Majoriti produk komersil yang diperolehi di pasaran diperolehi dalam bentuk ***E. coli***. Pengeluaran ***E. coli*** yang tinggi adalah dalam bentuk jasad rangkuman (IB) yang perlu melalui proses pelipatan yang mahal dan prosedur penulenan yang sukar untuk mengeluarkan lipopolisakarida (endotoksin). Sebagai kontras, rekombinan IFN- yang dihasilkan dari strain ***L. lactics*** dengan status GRAS tidak membentuk IB dan dapat mengeluarkan IFN pada kadar mukosa serta tidak memerlukan kos yang tinggi untuk proses lipatan dan penulenan. Ia juga boleh digunakan sebagai adjuvan untuk pembentukan vaksin baru bagi menentang jangkitan virus. Dalam kajian ini, empat rekombinan ***L. lactis***, iaitu MGIF (yang

mengandungi P32, sejenis promoter juzukan), PNZ, PNHIF, PNZUSPIF (yang mengandungi Pnis, sejenis promoter teraruhan) dihasilkan untuk pengeluaran IFN-alph 2b. Penghasilan IFN- 2b disahkan melalui ELISA dan Western blot. Ujian stabiliti plasmid menunjukkan bahawa plasmid rekombinan adalah stabil di dalam strain walaupun selepas 100 generasi. Kesan pelbagai sumber karbon (glukosa, sukrosa dan laktosa), induksi nisin dan masa inkubasi dalam medium M17 terhadap jumlah penghasilan produk telah dikaji. Hasil kajian menunjukkan bahawa penghasilan yang tertinggi dicapai dengan penggunaan glukosa untuk kesemua strain rekombinan dan kepekatan nisin untuk induksi strain rekombinan dengan promoter Pnis adalah 30 ng/mL. Sebagai tambahan, pengeluaran IFN tertinggi untuk rekombinan MG1363 (MGIF) adalah dengan tempoh inkubasi selama 9 jam and untuk rekombinan NZ9000 (NZIF and NZUSPIF) selama 4.5 jam. Antara keempat-empat rekombinan tersebut, jumlah produksi tertinggi dalam keadaan optimum telah dicapai oleh rekombinan NZ9000 yang membawa gen SP_{usp45}- IFN- 2b (~0.27 g/L). IFN yang terhasil dikenakan ujian bioaktiviti dan ia menunjukkan kadar bioaktiviti yang dapat diterima pada 1.9×10^6 IU/mg. Sebagai kesimpulan, keputusan kajian membuktikan IFN-alpha 2b dapat dihasilkan dalam ***L. Lactis*** pada paras kadar bioaktiviti yang dapat diterima.

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I certify that a Thesis Examination Committee has met on 1 February 2013 to conduct the final examination of Omid Bayat on his thesis entitled "Production Of Alpha-Interferon In ***Lactococcus Lactis***" in accordance with the 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.

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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 the Master of Science. The members of the supervisory committee were as follows:

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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 or concurrently submitted for any degree at Universiti Putra Malaysia or at any other institutions.

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OMID BAYAT

Date: 1 February 2013

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