UNIVERSITI PUTRA MALAYSIA

CLONING AND IMMUNOLOGICAL CHARACTERIZATION OF RECOMBINANT *VIBRIO CHOLERAE* O-ANTIGEN TRANSPORT PROTEIN EXPRESSED IN *LACTOCOCCUS LACTIS*

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By

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CLONING AND IMMUNOLOGICAL CHARACTERIZATION OF RECOMBINANT *Vibrio cholerae* O-ANTIGEN TRANSPORT PROTEIN EXPRESSED IN *Lactococcus lactis*

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The food grade *Lactococcus lactis* is a potential vehicle for protein delivery via the oral route. This study used lactococcal strains as models for producing the *wzm* gene that codes for the porin protein involved in transport of *Vibrio cholerae* lipopolysaccharide O-antigen. The 750 bp gene fragment was PCR-amplified from *Vibrio cholerae* O1 clinical isolates and cloned into the *L. lactis* nisin-controlled gene expression vector pNZ8048. The constructs were electrotransformed into *L. lactis* NZ9000 host strains where transcription of the gene on the RNA level was confirmed by reverse transcriptase PCR. Sequence comparison and multiple alignment of the translated cDNA nucleotides with that of known proteins reveal the presence of conserved structural domains of the ABC-2 membrane superfamily integral membrane protein component. Due to its hydrophobic

nature, whole cell *L. lactis* protein extract was subjected to solubilisation with the detergents sodium dodecyl sulfate (SDS) and Triton X-100 before being separated on SDS-PAGE and analysed on Western blot. In the case of the current study, solubilisation using SDS was found to be more efficient when compared to Triton X-100 in retrieving the expressed ≈ 34 kDa *wzm* porin as observed upon western blot analysis.

ELISA readings showed that oral administration of recombinant *L. lactis* into New Zealand White rabbits elicited a statistically significant increase of both IgG and IgA levels ($P < 0{\cdot}05$) when compared to the control group given only the preparation buffer. Challenge study with virulent *V. cholerae* O1 strains via the oral route evoked watery diarrhoea in rabbits given only the buffer throughout the immunization period, but fecal passing of both the recombinant and non-recombinant *L. lactis* groups were normal. This indicates a positive effect of the Lactococcal cells itself, probably towards the intestinal microbiota, in protecting against the adverse effects of *V. cholerae* and in evading diarrhoea. The diarrhoea lasted approximately two days in the control group, while the others were observed to be diarrhoea-free until the end of the study.

Bioinformatics and molecular methods have enabled prediction and detection of the *wzm* protein product which, as shown in this study, possesses the potential to elicit antibody production and enhance immunity. Administration of the *L. lactis* bacterium through the oral route was shown to increase mucosal immunity and assist in conferring protection against the diarrhoeal-causing disease cholera. These results provide more insight into the relatively unknown product of *V. cholerae* wzm, while providing a potential alternative

for health improvement against cholera that could be further developed for a safer, convenient, and effective method in protection and prevention of this disease.

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

PENGKLONAN DAN PENCIRIAN KEIMUNAN PROTEIN PENGANGKUT O-ANTIGEN *Vibrio cholerae* REKOMBINAN YANG DIEKSPRES DALAM *Lactococcus lactis*

Oleh

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Lactococcus lactis bergred makanan berpotensi untuk penghantaran protin secara oral. Kajian ini menggunakan stren-stren *Lactococcus* sebagai model untuk menghasilkan gen *wzm* yang mengkod untuk protin porin yang terlibat dalam pengangkutan O-antigen lipopolisakarida *Vibrio cholerae*. Cebisan gen 750 bp ini telah digandakan melalui PCR daripada isolat klinikal *Vibrio cholerae* O1 dan diklon ke dalam vektor pengekspres gen yang dikawal nisin, pNZ8048. Konstruk terhasil telah dielektrotransformasikan ke dalam stren hos *L. lactis* NZ9000 di mana transkripsi gen pada tahap RNA disahkan melalui PCR transkrip terbalik. Perbandingan jujukan serta penjajaran berbilang nukleotida cDNA yang diterjemah dengan protin-protin yang diketahui jujukannya menunjukkan kehadiran domain struktur terpelihara komponen protin membren integral dari keluarga membren ABC-2. Disebabkan sifat gerun airnya, ekstrak protin *L. lactis* telah dilarutkan dengan detergen sodium dodecyl sulfate (SDS) dan Triton X-100 sebelum diasingkan

melalui SDS-PAGE dan dianalisa dengan Western blot. Dalam kes kajian ini, larutan menggunakan SDS didapati lebih berkesan berbanding Triton X-100 dalam mendapatkan porin wzm ≈ 34 kDa yang diekspres seperti yang diperhatikan dari analisa Western blot.

Bacaan ELISA menunjukkan bahawa *L. lactis* rekombinan yang diberi secara oral kepada arnab-arnab New Zealand White menyebabkan kenaikan tahap IgG dan IgA yang ketara dari segi statistik ($P < 0.05$) bila dibandingkan dengan kumpulan kawalan yang hanya diberi buffer penyediaan tanpa bakteria. Ujikaji cabaran dilakukan dengan memberikan stren *V. cholerae* O1 secara oral menyebabkan cirit-birit berair kepada arnab-arnab yang hanya diberi buffer penyediaan sepanjang tempoh imunisasi, tetapi bahan buangan kedua-dua kumpulan yang diberi *L. lactis* biasa dan rekombinan adalah normal. Ini menandakan kesan positif sel-sel *Lactococcus*, kemungkinan terhadap mikrobiota usus, dalam melindungi daripada kesan-kesan negatif *V. cholerae* serta mengelak daripada cirit-birit. Cirit-birit ini berlangsung selama dua hari dalam kumpulan kawalan yang diberi buffer, sementara yang lain didapati bebas dari jangkitan sehingga tamat kajian.

Bioinformatik serta pengkaedahan molekul telah membolehkan ramalan dan pengesanan produk protin *wzm* yang seperti ditunjukkan dalam kajian ini mempunyai potensi untuk mencetus penghasilan antibodi dan meningkatkan tahap imun. Pemberian *L. lactis* secara oral telah menunjukkan peningkatan imun mukosa dan membantu dalam memberikan perlindungan terhadap penyakit kolera yang menyebabkan cirit-birit. Keputusan-keputusan kajian ini memberikan maklumat yang lebih mendalam berkaitan produk *wzm V. cholera* yang agak kurang dikenali, sekaligus menyediakan alternatif berpotensi bagi

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I certify that a Thesis Examination Committee has met on 6 July 2011 to conduct the final examination of Hana Farizah binti Zamri on her thesis entitled "Cloning and Immunological Characterization of Recombinant *Vibrio cholerae* O-antigen Transport Protein Expressed 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 degree of Master of Science.

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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, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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HANA FARIZAH ZAMRI

Date: 6 July 2011

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