UNIVERSITI PUTRA MALAYSIA

POTENTIAL ANTI-CANCER PROPERTIES OF BACTERIOCIN UL4 FROM *Lactobacillus plantarum* IN RATS INDUCED WITH COLON CANCER

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By

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

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Chairman : Latifah Saiful Yazan, PhD

Faculty : Institute of Bioscience

Colorectal cancer (CRC) is the fourth and the third most common cancer in men and women worldwide, respectively. Despite improvements in the treatment modalities of CRC such as surgery, radiotherapy and chemotherapy, all of them especially chemotherapy can result in severe side effects. Thus, more specific and effective treatment needs to be developed to improve and add to the available ones. Currently, much attention has been directed towards the development of natural or natural product-based anticancer agents that are believed to have lesser side effects. Among those are probiotics and their metabolites, and prebiotics. Bacteriocin UL4 (UL4) is a metabolite from *Lactobacillus plantarum* that showed antitumor promoting activities in mice induced with skin cancer. It was also demonstrated that postweaning rats fed with UL4 had a lower blood cholesterol concentration. This study was carried out not only to determine the anti-colorectal cancer properties of UL4, but also its immunomodulatory activities. Briefly,

Sprague Dawley male rats were injected subcutaneously with azoxymethane (AOM) for two subsequent weeks (15mg/kg/week) to induce colorectal cancer. The animals were fed with different percentages of UL4 (0.25%, 0.5% and 0.75% of UL4 (w/w)) added into drinking water at week 26, once daily for 12 weeks. The positive (with cancer) and negative control group (normal rats, without cancer) were also included. Upon completion, the animals were sacrificed. The colon, spleen and thymus were removed. Immune cell suspensions were prepared from spleen and thymus of rats. The regional distribution of aberrant crypt foci (ACF) at the proximal, medial and distal part was histopathologically evaluated following hematoxylin and eosin staining. In further classification of ACF into hyperplasia without dysplasia, mild to moderate dysplasia and severe dysplasia, the number of ACF in all classifications reduced significantly in group treated with 0.5 and 0.75% of UL4 as compared to the PC group. The incidence of tumor was also found to decrease significantly in all UL4-treated groups as compared to the PC group ($p>0.05$). In concordance with the reduced incidence of ACF and tumor, expression of ß-catenin decreased significantly in all UL4-treated groups as compared to PC group. Results showed that effects of UL4 on the incidence of tumor and on the expression of ß-catenin were dose-independent. The immunomodulatory properties of UL4 were determined based on the level of several cytokines (IFN-γ, TNF-α, IL-12 and IL-5). The level of studied cytokines (IFN-γ, TNF-α and IL-5) except for IL-12 in serum, increased significantly ($p<0.05$) in all UL4-treated groups as compared to the PC group. In general, the level of cytokines studied (IFN-γ, TNF-α, IL-12 and IL-5) increased significantly in groups treated with 0.5% and 0.75% of UL4 in both spleen and thymus cells as

compared to the PC group. Effects of UL4 on the level of studied cytokines seem to be dose-independent. As a conclusion, UL4 reduced the number of tumor and the expression of β-catenin in rats induced with colorectal cancer. UL4 also enhanced the production of IFN-γ, TNF-α, IL-12 and IL-5, the cytokines that are crucially involved and play a significant role in inhibition of colon carcinogenesis. Therefore, UL4 has potential as an anti-colorectal cancer agent possibly by modulating the immune responses of patients to fight cancer.

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

POTENSI CIRI-CIRI ANTI KANSER BACTERIOCIN UL4 DARIPADA *Lactobacillus plantarum* DALAM TIKUS YANG DIARUH DENGAN KANSER USUS BESAR

Oleh

NORAINA BINTI MUHAMAD ZAKUAN

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Kanser usus besar (CRC) adalah kanser keempat dan ketiga paling biasa di kalangan lelaki dan wanita di dunia, masing-masing. Disebalik penambahbaikan dalam modus rawatan CRC seperti pembedahan, radioterapi dan kemoterapi, kesemua rawatan tersebut terutamanya kemoterapi boleh menyebabkan kesan sampingan yang teruk seperti cirit-birit dan muntah yang teruk, penindasan tulang dan keguguran rambut pada pesakit. Oleh itu, rawatan yang lebih spesifik dan efektif perlu dibangunkan bagi memperbaiki dan menambah rawatan yang sedia ada. Baru-baru ini, lebih perhatian diarahkan kepada penghasilan agen anti-kanser semulajadi atau yang berasaskan semulajadi yang dipercayai mempunyai kesan sampingan yang kurang. Antaranya adalah probiotik dan hasil metabolit mereka, dan prebiotik. Bakteriosin UL4 (UL4) ialah metabolit daripada *Lactobacillus*

plantarum yang menunjukkan aktiviti anti-penggalak tumor dalam mencit yang diaruhkan kanser kulit. Ia juga menunjukkan bahawa tikus pascapenyapihan yang diberi UL4 mempunyai kepekatan kolesterol darah yang lebih rendah. Kajian ini dijalankan bukan hanya untuk menentukan ciri-ciri anti-kanser usus besar UL4, tetapi juga ciri-ciri modulatori imunnya. Secara ringkasnya, tikus jantan *Sprague Dawley* telah disuntik secara subkutaneus dengan azoxymethane (AOM) untuk 2 minggu berturut-turut (15mg/kg/minggu) untuk mengaruh kanser usus besar. Tikus kemudiannya diberi minum UL4 dengan peratusan yang berbeza (0.25%, 0.5% dan 0.75% UL4 (w/w)) pada minggu ke 26, sekali sehari selama 12 minggu. Kumpulan kawalan positif (dengan kanser) dan kumpulan kawalan negatif (tikus normal, tanpa kanser) turut dimasukkan. Pada akhirnya, tikus dikorbankan. Kolon, limpa dan timus tikus diambil. Ampaian sel imun daripada limpa dan timus tikus disediakan. Pengagihan fokus kripta aberan (ACF) mengikut bahagian iaitu proksimal, medial dan distal telah dinilai secara histopatologi berikutan pewarnaan dengan hematoksilin dan eosin. Dalam klasifikasi lanjutan kepada hiperplasia tanpa displasia, displasia sedikit hingga sederhana dan displasia yang teruk, bilangan ACF bagi ketiga-tiga pengelasan berkurang secara signifikan dalam kumpulan yang dirawat dengan 0.5% dan 0.75% UL4 berbanding kumpulan PC. Kejadian tumor juga didapati berkurang secara signifikan dalam kesemua kumpulan rawatan UL4 berbanding kumpulan PC ($p<0.05$). Selaras dengan pengurangan kejadian tumor dan penzahiran β-katenin berkurang secara signifikan di kesemua kumpulan rawatan UL4 berbanding kumpulan PC. Keputusan menunjukkan kesan UL4 terhadap kejadian tumor dan penzahiran β-catenin tidak bergantung kepada dos. Ciri-ciri modulatori imun UL4 ditentukan berdasarkan

kepada aras beberapa sitokin (IFN-γ, TNF-α, IL-12 dan IL-5). Aras sitokin yang dikaji (IFN-γ, TNF-α dan IL-5) kecuali IL-12 dalam serum, meningkat secara signifikan ($p<0.05$) dalam kesemua kumpulan rawatan UL4 berbanding kumpulan PC. Secara amnya, aras sitokin yang dikaji (IFN-γ, TNF-α, IL-12 dan IL-5) meningkat secara signifikan dalam kumpulan yang dirawat dengan 0.5% dan 0.75% UL4 dalam kedua-dua sel limpa dan timus berbanding kumpulan PC. Kesan UL4 ke atas aras sitokin yang dikaji kelihatan tidak bergantung kepada dos. Sebagai kesimpulan, UL4 mengurangkan bilangan tumor dan penzahiran β-katenin dalam tikus yang diaruh dengan kanser usus besar. UL4 juga didapati meningkatkan penghasilan IFN-γ, TNF-α, IL-12 dan IL-5, sitokin yang terlibat secara kritikal dan memainkan peranan yang signifikan dalam perencatan karsinogenesis usus besar. Oleh itu, UL4 dilihat berpotensi sebagai agen anti-kanser usus besar berkemungkinan melalui modulatori sistem imun untuk melawan kanser.

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I certify that an Examination Committee has met on date of viva voce to conduct the final examination of Noraina binti Muhamad Zakuan on her Master of Science thesis entitled "**Potential Anti-Cancer Properties of Bacteriocin UL4 from *Lactobacillus plantarum* in Rats Induced with Colon Cancer"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were 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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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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NORAINA BINTI MUHAMAD ZAKUAN

Date : 21 October 2011

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