



UNIVERSITI PUTRA MALAYSIA

**CYTOTOXIC AND ANTIPROLIFERATIVE PROPERTIES OF
METABOLITES PRODUCED BY SIX STRAINS OF LACTOBACILLUS
PLANTARUM ON HUMAN CANCER CELLS**

CHUAH LI OON

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METABOLITES PRODUCED BY SIX STRAINS OF *LACTOBACILLUS*
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By

CHUAH LI OON

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Chairman: Assoc. Prof. Dr. Foo Hooi Ling, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Whole cells, cytoplasmic fractions and fermented products of LAB have been tested for anticancer effect. However, limited information is available for the metabolites produced by *Lactobacillus plantarum*. In this study, the anticancer effect of metabolites produced by six strains of *L. plantarum* (UL4, TL1, RS5, RG14, RG11 and RI11) isolated from Malaysian fermented foods was evaluated. All metabolites exhibited *in vitro* cytotoxic effect on the tested cancer cells (breast, colorectal, cervical, liver and leukemia cancer cell lines). An increased cytotoxic effect was observed with increased dose of metabolites used and time of incubation. In particular, metabolites UL4 exerted the most potent cytotoxicity against human breast carcinoma cells MCF-7 in a dose- and time-dependent manner in MTT assay, with inhibition concentration of 50 % growth (IC₅₀) value of 15, 12 and 10% (v/v) for 24, 48 and 72 hours of incubation, respectively. In contrast, no cytotoxicity was detected in primary human peripheral blood mononuclear cells, mouse splenocytes, thymocytes and bone marrow cells for all the six metabolites tested. However,



limited cytotoxicity was detected in nonmalignant human glandular epithelium cells MCF-10A when treated with UL4 and RG14 metabolites. Additionally, UL4 metabolites did not cause haemolysis, indicating cytotoxic effect of metabolites of six strains of *L. plantarum* is selective for malignant cells but spared on normal cells.

Antiproliferative effect was focused on MCF-7 and colon cancer cell line (HT-29). In BrdU cell proliferation assay, all tested metabolites inhibited DNA synthesis of MCF-7 and HT-29 cells. An increased antiproliferative effect was observed with increased dose of metabolites used and time of incubation. In particular, UL4 metabolites exhibited 100% proliferation inhibition on MCF-7, whereas RG14 metabolites exhibited 89% proliferation inhibition on HT-29 for 72 hours of incubation. Growth arrest study showed significant cell growth inhibition ($P < 0.05$) in MCF-7 treated with UL4 metabolites and HT-29 cells treated with RG14 metabolites.

Mode of cell death induced by UL4 metabolites on MCF-7 cells was elucidated. Results obtained in trypan blue dye exclusion assay suggested that UL4 metabolites did not cause necrosis. Induction of apoptosis rather than necrosis by UL4 metabolites was evident by the presence of most characteristics of apoptosis such as cell shrinkage, blebbing of cell membrane and fragmentation of DNA and nucleus. Annexin V/PI staining showed that substantial early apoptotic cells were detected in MCF-7 cells treated with UL4 metabolites compared to untreated control group. Cells treated with UL4 metabolites showed growth arrest at G_0/G_1 cell phase at 24 hours, followed by the increment of cells in sub- G_0/G_1 in DNA cell cycle analysis. In addition, the TUNEL assay showed that remarkable TUNEL-positive cells were detected in UL4

metabolites-treated MCF-7 cells. The results obtained in this study indicate the potential use of LAB metabolites as a promising antiproliferative and apoptosis induction agent as an alternative in nutraceutical industry and cancer therapy.

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

**SIFAT-SIFAT SITOTOSIK DAN ANTI-PROLIFERASI METABOLIT YANG
DIHASILKAN OLEH ENAM STRAIN *LACTOBACILLUS PLANTARUM*
TERHADAP SEL KANSER MANUSIA**

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Keseluruhan sel, fraksi sitoplasma dan produk penapaian LAB telah diuji kesan antikanser. Namun, maklumat terhad didapati bagi metabolit yang dihasilkan oleh *Lactobacillus plantarum*. Dalam kajian ini, kesan antikanker metabolit berasal dari enam strain *L. plantarum* (I-UL4, TL1, RS5, RG14, RG11 dan RI11) dipencilkan dari makanan tertapai di Malaysia telah dinilai. Semua metabolit mempamerkan kesan sitotoksik secara *in vitro* pada sel-sel kanser diuji (sel kanser payudara, kolorektal, leher rahim, hati dan leukemia). Kesan sitotosik meningkat dengan penambahan dos metabolit dan masa inkubasi yang digunakan. Secara khususnya, metabolit UL4 mempamerkan sitotoksiti yang paling poten terhadap sel kanser payudara manusia MCF-7 dalam ujian MTT, bergantung kepada dos dan masa yang digunakan, dengan nilai kepekatan perencatan 50% pertumbuhan (IC_{50}) sebanyak 15, 12 dan 10% (v/v) selepas 24, 48 dan 72 jam inkubasi masing-masing. Sebaliknya, sitotoksiti tidak dikesan di sel mononuklear darah periferi manusia, sel spleen tikus, sel timus tikus dan sel sumsum tulang tikus untuk semua enam metabolit yang diuji.

Namun, sitotoksitas yang terhad dikesan di sel epitelium kelenjar manusia yang bukan malignant (sel MCF-10A) ketika dirawat dengan metabolit UL4 dan RG14. Secara tambahan pula, metabolit UL4 tidak menyebabkan hemolisis, menunjukkan kesan sitotoksik metabolit yang dihasilkan oleh enam strain *L. plantarum* adalah selektif untuk sel-sel yang malignant sahaja dan tidak memberi kesan terhadap sel normal.

Kesan anti-proliferasi yang tertumpu pada sel MCF-7 dan sel kanser usus besar manusia (sel HT-29) telah diuji. Dalam ujian proliferasi sel BrdU, kesemua enam metabolit yang diuji menunjukkan kesan anti-proliferasi terhadap sel MCF-7 dan sel HT-29. Kesan anti-proliferasi meningkat dengan peningkatan dos metabolit dan masa inkubasi yang digunakan. Secara khususnya, metabolit UL4 mempamerkan 100% perencatan proliferasi terhadap sel MCF-7, manakala metabolit RG14 mempamerkan 89 % perencatan proliferasi terhadap sel HT-29 pada tempoh incubasi 72 jam. Kajian penyekatan pertumbuhan menunjukkan perencatan pertumbuhan yang nyata ($P < 0.05$) dalam sel MCF-7 yang dirawat oleh metabolit UL4 dan sel HT-29 yang dirawat oleh RG14.

Cara kematian sel yang dirangsang oleh metabolit UL4 terhadap sel MCF-7 telah dinilai. Keputusan dalam ujian eksklusi tripan biru mencadangkan bahawa metabolit UL4 tidak menyebabkan nekrosis. Induksi apoptosis dan bukan nekrosis oleh UL4 metabolit terbukti dengan pengesanan ciri-ciri utama apoptosis seperti pengecutan sel, “membrane blebbing” serta frakmentasi DNA dan nukleus. Dalam sel MCF-7 yang dirawat dengan metabolit UL4, sel apoptotic awal yang ketara dikesan dalam ujian perwarnaan annexin V/PI berbanding dengan kumpulan kawalan yang tidak dirawat. Dalam analisis kitaran

sel DNA, sel yang dirawat dengan metabolit UL4 menunjukkan penyekatan pertumbuhan di fasa sel G_0/G_1 dalam tempoh 24 jam, diikuti dengan penambahan sel dalam fasa sub- G_0/G_1 . Tambahan pula, penyelidikan fragmentasi DNA dengan ujian TUNEL menunjukkan bahawa sebahagian besar sel yang positif dalam ujian TUNEL telah dikesan dalam sel MCF-7 yang dirawat dengan metabolit UL4. Keputusan yang diperolehi dalam kajian ini menunjukkan potensi terjanji penggunaan metabolit UL4 sebagai satu agen sitotoksik dan perangsang apoptosis, dan boleh digunakan sebagai alternatif industri nutraseutikal dan rawatan kanser.

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DECLARATION

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

CHUAH LI OON

Date: 9 December 2010

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