UNIVERSITI PUTRA MALAYSIA

INVESTIGATION OF THE INTERACTION BETWEEN LACTOCOCCUS LACTIS M4 AND HUMAN COLORECTAL CANCER CELL LINES, SW620

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FBSB 2015 149

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ABSTRACT

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement for the degree of B.Sc Cell and Molecular Biology.

INVESTIGATION OF THE INTERACTION BETWEEN LACTOCOCCUS LACTIS M4 AND HUMAN COLORECTAL CANCER CELL LINES, SW620

By

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June 2015

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Lactic Acid Bacteria (LAB) are frequently used in food fermentation and probiotic dairy products. They are known to be beneficial to the human gut microflora have a Generally Regarded as Safe (GRAS) status. In recent years, there has been a resurgence of interests of scientists to develop LAB as therapeutic vaccine carriers to target cancer cells. To assess the potential of LAB as a DNA delivery vehicle, interactions of the LAB strains with the cancer cell lines need to be investigated. The interactions of a local dairy strain, *Lactococcus lactis* M4 was compared with a chicken intestine derived reference strain, *Lactobacillus spp.* CI314 against human colorectal cancer cell lines SW620 at multiplicity of infection (MOI) 250 bacteria per cell. Both rate of adhesion and invasion of M4 strain were significantly higher than CI314 strain. In an alternative adhesion assay using crystal violet staining, M4 strain had also shown a significantly higher adhesion rate when compared to CI314. However, interaction assay at higher MOI (500:1) of M4 strain shows cytotoxicity effect towards SW620 cell lines when compared to the control cells. Cell viability assessment via trypan blue counting had shown a low cell recovery rate, suggesting SW620 cell detachment from the monolayer. Based on the promising invasion and adhesion results from the interaction assay, it can be concluded that *L. lactis* M4 is a potential candidate to be developed into a DNA delivery vehicle.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhikan keperluan untuk ijazah B.Sc Biologi Sel dan Molekul.

KAJIAN INTERAKSI ANTARA LACTOCOCCUS LACTIS M4 DAN SEL KANSER KOLOREKTAL MANUSIA, SW620

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Bakteria laktik asid (LAB) sering digunakan dalam fermentasi makanan dan produk susu probiotik. LAB dikenali sebagai bakteria yang boleh memberi manfaat kepada mikroflora usus manusia dan mempunyai status “Dianggap Selamat Secara Umumnya” (GRAS). Sejak kebelakangan ini, ramai penyelidik berminat untuk membangunkan LAB sebagai pembawa vaksin terapeutik terhadap sel-sel kanser. Untuk mengeksplorasi potensi LAB sebagai pembawa DNA, interaksi LAB dengan sel-sel kanser perlu dikaji. Interaksi sejenis LAB yang dipencilkan daripada susu tempatan, *Lactococcus lactis* M4 telah dibandingkan dengan *Lactobacillus spp.* CI314 yang diperolehi daripada usus ayam terhadap sel kanser kolorektal manusia, SW620. Pada gandaan jangkitan (MOI) 250 bakteria per sel, kadar lekatan dan kemasukan M4 lebih tinggi berbanding CI314. Dalam suatu kajian lekatan alternatif melalui pewarnaan kristal ungu, M4 juga mempunyai kadar lekatan lebih tinggi berbanding dengan CI314. Pada MOI yang tinggi (500:1), M4 mempunyai kesan sitotoksik terhadap sel SW620 apabila dibanding dengan sel kontrol. Kajian viabiliti melalui pengiraan sel dengan Trypan biru menunjukan bahawa bilangan sel hidup yang diperoleh adalah amat rendah. Ini mungkin disebabkan oleh penanggalan sel SW620 dari lapisan sel. Oleh sebab kadar pelekatan dan kemasukan M4 amat memuaskan dalam kajian-kajian yang telah dijalankan, M4 berpotensi dibangunkan sebagai pembawa DNA.

ACKNOWLEDGEMENTS

First and foremost, I would like to extend my greatest and deepest gratitude to my supervisor, Dr. Siti Sarah Othman for her constructive suggestions, patience and review of my work throughout the completion of this study despite her busy schedule. My warmest gratitude also goes to Assoc. Prof. Dr Janna Ong Abdullah for her guidance given as the coordinator of the course BSM 4999.

Furthermore, I would like to thank Miss Habibah Faroque for her guidance, assistance and knowledge sharing throughout the study. Special thanks extended to all members of the Microbial Biotechnology lab for sharing the facilities with me, and especially when they had to stay back to lock the lab when my experiments ran late on several occasions.

I would also like to thank Mr. Loo Shu San for his collaboration, supervision and permission for me to work in his lab in Agro-Biotechnology Institute Malaysia (ABI) and Miss Wan Nur Fadhilah Shamsudin for her patience in supervising my work and various sacrifices to fit me into her busy schedule.

Last but not least, I would like to express my gratitude to my parents and close friends, especially Lam Wai Liam and Lau Kah Cheong for their care and support throughout this challenging semester. This research project would not be possible without the help of all of the parties mentioned above. My sincerest thanks to each and every one of you!

APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfillment of the requirement for the degree of B.Sc Cell and Molecular Biology. The member of the supervisory committee was as follows:

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DECLARATION

Declaration by undergraduate student

I hereby confirm that:

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This is to confirm that:

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Name of

Chairman of

Supervisory Committee: _____

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LIST OF ABBREVIATIONS

^{o}C	Degree Celsius
%	Percent
Caco-2	Human colorectal adenocarcinoma cells, Caco-2
CFU	Colony forming units
CI314	*Lactobacillus spp.* CI314
LAB	Lactic acid bacteria
µg	Microgram
µL	Microliter
µg/mL	Microgram per microliter
mL	Milliliter
M4	*Lactococcus lactis* M4
MOI	Multiplicity of infection
SEM	Standard error of the mean
SW620	Human colorectal cancer cell lines, SW620

CHAPTER 1

Introduction

Lactic Acid Bacteria (LAB) has been discovered and used by humans for more than 5000 years. They are generally well known for their usage in food fermentation throughout history and more recently in probiotic dairy products. They are gram-positive, acid-tolerant, and generally anaerobic bacteria in the shape of rods or cocci (Nikita & Hemangi, 2012). The LAB family includes the genera *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus,* and *Streptococcus* (Stiles & Holzapfel, 1997). In this project, two strains of LAB were used, namely *Lactococcus lactis* M4, which was a locally isolated dairy strain, and a reference strain, *Lactobacillus spp.* CI314, which is isolated from the intestines of local chicken. First and foremost, a major problem with using bacteria as anti-cancer agents is their toxicity at the dose required for therapeutic efficacy and reducing the dose ultimately results in diminished efficacy (Patyar et al., 2010). This would not be the case when LAB is used, since they are “Generally Recognized as Safe” (GRAS) and poses minimal risk to humans when used for therapeutic applications (Sybesma, Hugenholtz, De Vos, & Smid, 2006). In fact, they are beneficial to the human mucosal surfaces via formation of healthy microflora (Rafter, 2002). Moreover, there are a lot of widely used, effective commercially available expression systems such as the Nisin Controlled Gene Expression System (NICE) available (Mierau & Kleerebezem, 2005), combined with the fact that the genome of *L. lactis* has been extensively studied, sequenced and

characterized (Bolotin et al., 2001), hence making studies related to it considerably simpler and easier. It has been demonstrated that *L. lactis* M4, isolated from local dairy milk, despite being cultured for 100 generations, was able to survive on erythromycin supplemented media and express green fluorescence protein via an inserted plasmid (Noreen et al., 2011). It was also reported that M4 had low molecular weight plasmids, reducing the risk of plasmid incompatibility, making it a prime candidate as a potential host for the expression of heterologous proteins (Noreen et al., 2011). It was also reported in different studies of the success in using genetically engineered *L. lactis* in cancer research as DNA delivery vehicles. For instance, Li and colleagues reported a strain of recombinant *L. lactis* expressing recombinant rat endostatin protein, when administered orally to rats with 1,2-dimethylhydrazine (DMH) induced colorectal cancer, exhibited significant effect on colorectal cancer and the mean survival time of the rats treated with endostatin was longer (Li & Li, 2005). Another group of researchers reported an isolated strain of *L. lactis*, named 44Lac, which displayed cytotoxic properties via its secreted metabolites on cancer cell lines such as HT29, AGS, MCF-7, and HeLa. It was described that its performance was similar to that of Taxol against all the cell lines tested (Haghshenas, Nami, & Radiah, 2014). Besides that, based on reports by a group of Korean researchers that studied the anti-proliferative effects of cytoplasmic fraction of *L. lactis ssp. lactis* on SNU-1 human stomach cancer cell line, the cytoplasmic fraction of *L. lactis* caused G0/G1 cell cycle arrest and apoptosis in the SNU-1 cells (S. Y. Kim, Kim, Lee, & Joo, 2009). It can thus be

seen that LAB displayed very promising results in the field of cancer research. It is hypothesized that if M4 displays interactions with colon cancer cell lines, it may be potentially engineered to carry therapeutic vaccine to target cancer cells.

The particular objectives for this study were:

1. To investigate the adherence of *L. lactis* M4 towards human colorectal cell lines.
2. To investigate the invasion of *L. lactis* M4 towards human colorectal cell lines.

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