UNIVERSITI PUTRA MALAYSIA

MICROBIOTA PROFILE OF MALAYSIAN KEFIR GRAIN AND ANTI-METASTATIC EFFECTS OF KEFIR WATER IN MURINE BREAST CANCER CELLS

NUR RIZI ZAMBERI

FBSB 2016 33

MICROBIOTA PROFILE OF MALAYSIAN KEFIR GRAIN AND ANTI-METASTATIC EFFECTS OF KEFIR WATER IN MURINE BREAST CANCER CELLS

By

NUR RIZI ZAMBERI

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

June 2016

DEDICATION

This thesis is dedicated to my beloved parents, Mr. Zamberi and Mrs. Rohana,

who always picked me up on time

and encouraged me to go on every adventure,

especially this one.

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Master of Science

MICROBIOTA PROFILE OF MALAYSIAN KEFIR GRAIN AND ANTI-METASTATIC EFFECTS OF KEFIR WATER IN MURINE BREAST CANCER CELLS

By

NUR RIZI BINTI ZAMBERI

June 2016

Chairman : Assoc. Prof. Noorjahan Banu Alitheen, PhD
Faculty : Biotechnology and Biomolecular Science

Breast cancer is the leading cancer in women where metastasis is the major cause of breast cancer death. For years, a cure for cancer is still being researched on and the inhibition of metastasis cascade is one of the goals. The association of functional food such as probiotics with cancer therapy has become an emerging field of study. Kefir is a uniquely cultured product and traditionally used as a daily serving in Eastern Europe for its health benefits. Reports have shown that kefir has health-promoting benefits such as giving rise to antioxidants, stimulates immune response and possesses anti-tumor properties. Recently, it has gained popularity in Asian countries including Malaysia. This study aims to detect microbial diversity present in kefir grain using 16S metagenomic approach, evaluates its antioxidant potential and assesses the anti-metastatic effect of kefir towards murine breast cancer model *in vitro* and *in vivo*. The microbial diversity in kefir water was determined by using Next-Generation Sequencing (NGS) and antioxidant assays were performed to evaluate the antioxidant potential of kefir water. *In vitro* and *in vivo* anti-metastasis studies of 4T1 murine breast cancer cells were further investigated. *Lactobacillus* genus was the dominant genus detected in the kefir grain where the predominant species was *L. kefiranofaciens* while *L. kefiri* was the second in abundance. Kefir water displayed prominent antioxidant activities and gallic acid (0.816 ± 0.11 μg/mg) was detected in the HPLC profile. Kefir water was cytotoxic towards 4T1 cells *in vitro* and further *in vitro* assessment on metastasis was evaluated using wound healing assay, *in vitro* migration and invasion assay and mouse aortic ring assay. In the murine model, a significant reduction in tumor size and weight (0.91 ± 0.21 g) and a substantial increase in helper T cells (5-fold) and cytotoxic T cells (7-fold) were observed in the kefir water–treated group. The mechanism of action of kefir water was further demonstrated at the molecular level by conducting real time polymerase chain reaction and proteomic profiler. These findings suggest that kefir water contains largely *Lactobacillus* species and possesses antioxidant property. Kefir water inhibited tumor proliferation in vitro and in vivo mainly through cancer cell apoptosis, immunomodulation by stimulating T helper cells and cytotoxic T cells, and anti-inflammatory, antimetastatic, and antiangiogenesis effects. This study brought out the potential of the probiotic beverage kefir water in cancer treatment.

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

PROFIL MIKROBIOTA DARIPADA BIJIRIN KEFIR DI MALAYSIA DAN KESAN ANTI METASTASIS AIR KEFIR TERHADAP SEL KANSER PAYUDARA TIKUS

Oleh

NUR RIZI BINTI ZAMBERI

Jun 2016

Pengerusi : Prof. Madya. Noorjahan Banu Alitheen, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Kanser payudara adalah kanser yang paling utama dalam kalangan wanita dan metastasis adalah penyebab utama kematian kanser payudara. Setelah bertahun-tahun, penyembuh kepada kanser masih lagi dikaji dan salah satu matlamatnya adalah perencatan kepada urutan metastasis. Pertalian antara makanan pelbagai fungsi seperti probiotik dengan terapi kanser adalah bidang pengajian yang terkini. Kefir adalah produk kultur yang unik dan digunakan secara tradisional sebagai hidangan harian di Timur Eropah untuk manfaat kepada kesihatan. Laporan menunjukkan bahawa kefir telah menyumbang kepada pelbagai kebaikan kepada kesihatan seperti mempunyai antioksidan yang tinggi, merangsang tindak balas imun dan mempunyai ciri anti-barah. Pada ketika ini, ia telah mendapat populariti di negara-negara Asia termasuk Malaysia. Kajian ini bertujuan untuk mengesan kepelbagaian mikrob di dalam bijirin kefir menggunakan pendekatan 16S metagenomik, menilai potensi antioksidan air kefir dan juga menilai kesan anti-metastasis oleh air kefir terhadap model sel kanser payudara tikus *in vitro* dan *in vivo.* Kepelbagaian mikrob dalam bijirin kefir telah ditentukan dengan menggunakan Next Generation Sequencing (NGS) dan beberapa ujian antioksidan telah dijalankan bagi menentukan potensi antioksidan dari air kefir. Kajian anti-metastasis oleh air kefir terhadap sel kanser payudara 4T1 telah dikaji dengan lebih mendalam. Genus *Lactobacillus* adalah genus dominan yang dikesan di dalam bijiran kefir di mana spesies yang terbanyak adalah *L. kefiranofaciens* manakala *L. kefiri* adalah yang kedua terbanyak. Aktiviti antioksidan di dalam air kefir adalah memberangsangkan dan asid galik (0.816 ± 0.11 μg/mg) juga dikesan di dalam profil kromatografi cecair prestasi tinggi (HPLC) air kefir. Air kefir mempunyai kesan sitotosik terhadap sel-sel 4T1 *in vitro* dan penilaian lanjut pada kesan metastasis telah dinilai melalui analisis penyembuhan luka, analisis *in vitro* migrasi dan invasi dan juga analisis gelungan aortik tikus. Dalam model tikus, air kefir mengurangkan saiz dan berat tumor kanser payudara (0.91 ± 0.21 g) dan terdapat peningkatan besar dalam sel-sel T penolong (5 kali ganda) dan sel-sel T sitotoksik (7 kali ganda) di dalam kumpulan rawatan air kefir. Mekanisma tindakan oleh air kefir telah didemonstrasi pada tahap molekul dengan menjalani analisis rantaian polimerasi reaksi masa sebenar dan profil protin. Penemuan-penemuan ini menunjukkan bahawa air kefir mengandungi spesis *Lactobacillus,* mempunyai nilai antioksidan. Air kefir juga menghalang percambahan

tumor *in vitro* dan *in vivo* terutamanya melalui apoptosis sel kanser, tindak balas imunisasi dengan merangsang sel-sel T penolong dan sel-sel T sitotosik, dan ia juga mengandungi kesan anti-radang, anti-barah, dan anti-angiogenesi. Kajian ini menunjukkan bahawa air kefir probiotik ini mempunyai potensi sebagai rawatan penyakit kanser.

ACKNOWLEDGEMENT

To the greatest extend, I praise to Allah S.W.T as I am able to finish my Masters project as well as compiling this thesis. Special thanks to my parents and family for their love and constant support through the thick and thin of my journey.

I would like to extend my appreciation to my beloved supervisor, Assoc. Prof. Dr. Noorjahan Banu Alitheen for accepting me to be her student when I first approached her. She has provided me with guidance, constructive criticism, encouragement, understanding, and patience throughout my master's journey. A special thank to Dr. Yeap Swee Keong as my co-supervisor who has given me knowledgeable ideas and encouragement as well as guidance in my project research. My appreciation is also dedicated to my other supervisory committee members: Dr Nik Mohd Afizan and Prof. Zuki.

I would like to recognize my awesome, happening, and very helpful ATCL lab members who had taught me priceless things that I would not get in other places: Dr. Nadiah, Ms Elyani, Mrs. Noraini, Mr. Firdaus, Mrs. Roszaimah and to all my friends who come and go in ATCL lab.

I also thank Graduate Research Fellowship (GRF) from UPM, MyMaster15 from Ministry of Higher Education, and my father for their financial support until the end of my Masters journey.

Thank you, from the bottom of my heart.

I certify that a Thesis Examination Committee has met on 3 June 2016 to conduct the final examination of Nur Rizi binti Zamberi on her thesis entitled "Microbiota Profile of Malaysian Kefir Grain and Anti-Metastatic Effects of Kefir Water in Murine Breast Cancer Cells" 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.

Members of the Thesis Examination Committee were as follows:

Nor' Aini binti Abdul Rahman, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Janna Ong binti Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Datin Sri Nurestri Abd Malek, PhD
Professor
University of Malaya
Malaysia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 23 August 2016

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:

Noorjahan Banu Binti Mohammed Alitheen, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Yeap Swee Keong, PhD
Fellow Research
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Md Zuki Bin Abu Bakar @ Zakaria, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Nik Mohd Afizan Bin Nik Abdul Rahman, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Nur Rizi Binti Zamberi, GS37170

Declaration by Members of Supervisory Committee

This is to confirm that:

- this research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

LAB	Lactic Acid Bacteria
Th1	T-helper 1
Th2	T-helper 2
ER	Estrogen Receptors
EMT	Epithelial to masenchymal transition
HGF	Hepatocyte growth factor
VEGF	Vascular endothelial growth factor
PDGF	Platelet derived growth factor
MMP	Matrix metalloproteases
LPS	Lipopolysaccharide
dsRNA	Double stranded RNA
DNA	Deoxyribonucleic acid
ROS	Reactive oxygen species
NO	Nitric oxide
DCs	Dendritic cells
NK	Natural killer
APC	Antigen-presenting cells
TILs	Tumor-infiltrating lymphocytes
GALT	Gut-associated lymphoid tissue
FAE	Follicle-associated epithelium
M cells	Microfold cells
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
NGS	Next generation sequencing
O_2^-	Superoxide
HO	Hydroxyl
ROO	Peroxyl
Alkoxyl	RO
ORAC	Oxygen radical antioxidant capacity
DPPH	1, 1-Diphenyl -2-picryl-hydrazyl

GSH	Total glutathione
TPC	Total phenolic content
TF	Total flavonoid content
cDNA	Complementary DNA
PBS	Phosphate buffer saline
CTAB	Cetyltrimethylammonium bromide
EDTA	Ethylenediaminetetraacetic acid
NaCl	Sodium chloride
RNAse	ribonuclease
TE	Tris-EDTA
NCBI	National Centre for Biotechnology Information
BLAST	Basic local alignment search tool
MEGAN	"MEtaGenome Analyzer
LCA	Lowest common ancestor
DMSO	Dimethyl sulfoxide
FRAP	Ferric reducing antioxidant power
HCL	Hydrochloric acid
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
$AlCl_3$	Aluminium chloride
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
DTNB	69-78-3; 5,5'-Dithiobis(2-nitrobenzoic acid)
NADPH	Nicotinamide adenine dinucleotide phosphate
HPLC	High performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration
NH_4Cl	Ammonium chloride
Na_2EDTA	Disodium salt of ethylenediaminetetraacetic acid
KH_2PO_4	Monopotassium phosphate
HRP	Horsedish peroxidase
DAB	3,3'-Diaminobenzidine
IL-2	Interleukin-2
IFN-γ	Interferon-gamma
IL-10	Interleukin-10

IL-1β	Interleukin-1 beta
MDA	Malondialdehyde
FACS	Fluorescence-activated cell sorter
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
ACTB	Beta actin
MMP-9	Matrix metallopeptidase 9
iNOS	Inducible nitric oxide synthase
ICAM1	Intercellular Adhesion Molecule 1)
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
TNF-α	Tumor necrosis factor alpha
IL-4	Interleukin-4
RIPA	Radioimmunoprecipitation assay
AAE	Ascorbic acid equivalent
TE	Trolox equivalent
AUC	Area under curve
H&E	Hematoxylin and eosin
ELISA	Enzyme-linked immunosorbent assay
TIMP-1	The tissue inhibitor of metalloproteinase-1
TIMP-2	The tissue inhibitor of metalloproteinase-2
PCR-DGGE	Polymerase chain reaction denaturing gradient gel electrophoresis
TNBC	Triple negative breast cancer
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling

CHAPTER 1

INTRODUCTION

Cancer is infamous as the leading cause of death in the world. In the United States, breast cancer is the most common type of cancer for women (Siege, Ma, Zou, & Jemal, 2014). One out of 20 woman in Malaysia has the tendency to develop breast cancer in their lifetime (Yip, Taib, & Mohamed, 2006). Cancer is caused by both internal factors such as inherited mutation and immune condition, and environmental factors such as diet and lifestyle (Anand et al., 2008). The majority of breast cancer death is mainly because of cancer metastasis to other parts of the body (Scully, Bay, Yip, & Yu, 2012). Metastasis is the relocation of a disease process from a primary site to a distant one by a movement of the causal agents through the blood vessels or lymph channels (Hoover & Ketcham, 1975). Metastasis is an event that includes several stages that subsequently contribute to the formation of secondary tumors in adjacent organs and nontransformed cells (Martin, Ye, Sanders, Lane, & Jiang, 2000). For years, a cure for cancer is still being researched on and the inhibition of metastasis cascade is one of the goals.

The association of functional food such as probiotics with cancer therapy has become an emerging field of study. In infectious disease prevention and therapy, probiotics help in modulating both innate and acquired host's defenses mechanism (Oelschlaeger, 2010). Probiotics refer to live microorganisms which when administered in adequate amounts confer a health benefit on the host (World Health Organization, 2006). Although the usage of probiotics as a therapeutic agent is most common in colon-related diseases (Altonsy, Andrews, & Tuohy, 2010; Le Leu et al., 2005) due to the ability of the bacteria to interact directly with the colon after consumption, it also has the anti-tumor properties in breast cancer cells (de Moreno de LeBlanc, Matar, Farnworth, & Perdigon, 2006).

Kefir was introduced over a thousand years ago in the Caucasus Mountains in Central Asia. It is a traditional beverage made from the fermentation of kefir grains with milk or water (Ot1es & Cagindi, 2003). The microbiota community present in kefir grains is varied depending on the source of the grains and its environment of culture (Vardjan, Mohar Lorbeg, Rogelj, & Čanžek Majhenič, 2013). Thus, it is essential to obtain an accurate microbial identification in a population to get insights of which specific organism is responsible as an etiological agent such as for diagnosis purposes as well as antimicrobial potential (Clarridge, 2004). Kefir grains contain various kinds of microbial flora including the most commonly studied species due to their probiotic potential such as *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactococcus lactis* subsp. *Lactis* (Kakisu, Irigoyen, Torre, De Antoni, & Abraham, 2011; Otles, Cagindi, & Akcicek, 2003). Since these species have numerous different strains, not every single strain has the potential to be probiotic. This important characteristic makes kefir suitable as a probiotic beverage. In Malaysia, people have started to acknowledge

the benefits of kefir and consume kefir as daily health supplement. However, up to date, there is no report made on the metagenomics of kefir grains obtained locally.

Kefir has been consumed extensively for years as it is believed to promote greater health and this claim was verified by recent studies in which kefir was shown to aid in alleviating lactose intolerance (De Vrese, Keller, & Barth, 1992), inflammation (Huseini, Rahimzadeh, Fazeli, Mehrazma, & Salehi, 2012; Rodrigues, Carvalho, & Schneedorf, 2005) and high total cholesterol (Huang et al., 2013; Wang et al., 2009), and exhibited immunomodulation properties (Vinderola, Duarte, Thangavel, Perdigón, Farnworth, & Matar, 2005a). It has also been reported that kefir has the ability to induce cytotoxicity and reduce cancer growth *in vivo* (de Moreno de LeBlanc et al., 2006; Shiomi, Sasaki, Murofushi, & Albara, 1982). Furthermore, studies by de Moreno de LeBlanc et al. (2006) on the administration of commercial milk kefir from Canada in breast cancer murine model determined that it can slow down cancer by elevating local immune response and selected cytokines (de Moreno de LeBlanc et al., 2006; de Moreno de LeBlanc, Matar, Farnworth, & Perdigón, 2007). Nevertheless, in-depth study is needed on the anti-tumor and anti-metastatic effects of kefir in murine breast cancer model.

Therefore, the objectives of this study are:

1) To detect microbial diversity present in kefir grain using 16S metagenomic approach.
2) To evaluate the antioxidant properties of kefir water.
3) To assess the anti-metastasis effects of kefir water towards murine breast cancer cell, 4T1, *in vitro.*
4) To investigate the anti-metastasis effects of kefir water towards 4T1 challenged BALB/c mice *in vivo.*

REFERENCES

Abbas, A. K., Lichtman, A. H. H., & Pillai, S. (2014). *Cellular and molecular immunology*. Elsevier Health Sciences.

Abu, N., Mohamed, N. E., Yeap, S. K., Lim, K. L., Akhtar, M. N., Zulfadli, A. J., Beh, B. K., Abdullah, M. P., Omar, A. R., Alitheen, N. B. (2015). In vivo antitumor and antimetastatic effects of flavokawain B in 4T1 breast cancer cell-challenged mice. *Drug Design, Development and Therapy, 9*, 1401-1417.

Adams, M. R., & Moss, M. O. (2008). *Food microbiology*. Cambridge: Royal Society of Chemistry.

Aggarwal, B. B. (2004). Nuclear factor-κB: The enemy within. *Cancer Cell, 6*(3), 203-208.

Ahmad, S. (2012). *Oxidative stress and antioxidant defenses in biology* Springer Science & Business Media.

Ahmed, Z., Wang, Y., Ahmad, A., Khan, S. T., Nisa, M., Ahmad, H., & Afreen, A. (2013). Kefir and health: A contemporary perspective. *Critical Reviews in Food Science and Nutrition, 53*(5), 422-434.

Albini, A., Iwamoto, Y., Kleinman, H. K., Martin, G. R., Aaronson, S. A., Kozlowski, J. M., & McEwan, R. N. (1987). A rapid in vitro assay for quantitating the invasive potential of tumor cells. *Cancer Research, 47*, 3239-3245.

Albuquerque, P., Mendes, M. V., Santos, C. L., Moradas-Ferreira, P., & Tavares, F. (2009). DNA signature-based approaches for bacterial detection and identification. *Science of the Total Environment, 12*, 3641-3651. doi:10.1016/j.scitotenv.2008.10.054

Altonsy, M. O., Andrews, S. C., & Tuohy, K. M. (2010). Differential induction of apoptosis in human colonic carcinoma cells (caco-2) by atopobium, and commensal, probiotic and enteropathogenic bacteria: Mediation by the mitochondrial pathway. *International Journal of Food Microbiology, 137*, 190-203.

Amaretti, A., di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M., & Bordoni, A. (2013). Antioxidant properties of potentially probiotic bacteria: In vitro and in vivo activities. *Applied Microbiol and Biotechnology, 2*, 809-817. doi:10.1007/s00253-012-4241-7

Anand, P., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., Sung, B., Aggarwal, B. B. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research, 25*(9), 2097-2116.

Anders, C. K., & Carey, L. A. (2009). Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. *Clinical Breast Cancer, 9*, S73-S81.

Aragón, F., Carino, S., Perdigón, G., & de LeBlanc, Alejandra de Moreno. (2014). The administration of milk fermented by the probiotic lactobacillus casei CRL 431 exerts an immunomodulatory effect against a breast tumour in a mouse model. *Immunobiology, 219*(6), 457-464.

Aslakson, C. J., & Miller, F. R. (1992). Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Research, 52*(6), 1399-1405.

Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y., & González-Aguilar, G. A. (2004). A. effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *LWT Food Science and Technology, 37*, 687-695. doi:10.1016/j.lwt.2004.03.002

Bae, S. Y., Han, J. H., Kim, S., Lee, S. K., Lee, J. H., Lee, H. C., Kim, M., Kim, J., Kil, W. H., Kim, S. W, Lee, J. E., Nam, S. J., (2013). Abstract P2-09-19: Zerumbone suppresses IL-1b-induced cell migration and invasion through inhibition of IL-8 expression and MMP3 expression in human triple negative breast cancer cells. *Cancer Research, 73*(24 Supplement), P2-09-19.

Baker, M., Robinson, S. D., Lechertier, T., Barber, P. R., Tavora, B., D'Amico, G., Jones, D. T., Vojnovic, B., Hodivala-Dilke, K. (2012). Use of the mouse aortic ring assay to study angiogenesis. *Nature Protocols, 7*(1), 89-104.

Baliga, M. S., Meleth, S., & Katiyar, S. K. (2005). Growth inhibitory and antimetastatic effect of green tea polyphenols on metastasis-specific mouse mammary carcinoma 4T1 cells in vitro and in vivo systems. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 11*(5), 1918-1927. doi:11/5/1918 [pii]

Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: Back to virchow? *The Lancet, 357*(9255), 539-545.

Batra, J., Robinson, J., Mehner, C., Hockla, A., Miller, E., Radisky, D. C., & Radisky, E. S. (2012). PEGylation extends circulation half-life while preserving in vitro and in vivo activity of tissue inhibitor of metalloproteinases-1 (TIMP-1). *PLOS One, 7*, e50028.

Benzie, I. F., & Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70-76.

Beyer, M., & Schultze, J. L. (2006). Regulatory T cells in cancer. *Blood, 108*(3), 804-811. doi:108/3/804

Bolotin, A., Quinquis, B., Renault, P., Sorokin, A., Ehrlich, S. D., Kulakauskas, S.,

Lapidus, A., Goltsman, E., Mazur, M., Pusch, G. D., Fonstein, M., Overbeek, R.,

Kyprides, N., Purnelle, B., Prozzi, D., Ngui, K., Masuy, D., Hancy, F., Burteau, S.,

Boutry, M., Delcour, J., Goffeau, A., Hols, P. (2004). Complete sequence and comparative genome analysis of the dairy bacterium streptococcus thermophilus. *Nature Biotechnology, 12*, 1554-1558. doi:10.1038/nbt1034

Bornman, D. M., Hester, M. E., Schuetter, J. M., Kasoji, M. D., Minard-Smith, A., Barden, C. A., Nelson, C. N., Godbold, D. G., Baker, H. C., Yang, B. (2012). Short-read, high-throughput sequencing technology for STR genotyping. *BioTechniques.Rapid Dispatches, 2012*, 1.

Brazma, A., & Vilo, J. (2000). Gene expression data analysis. *FEBS Letters, 480*(1), 17-24.

Bustin, S. A. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular Endocrinology, 25*(2), 169-193. doi:JME00927

Cahill, D. J. (2001a). Protein and antibody arrays and their medical applications. *Journal of Immunological Methods, 250*(1), 81-91.

Cahill, D. J. (2001b). Protein and antibody arrays and their medical applications. *Journal of Immunological Methods, 250*(1), 81-91.

Can, E., Kurtoğlu, İ. Z., Benzer, F., Erişir, M., Kocabaş, M., Kızak, V., Kayim, M., Çelik, H. T. (2012). The effects of different dosage of kefir with different durations on growth performances and antioxidant system in the blood and liver tissues of çoruh trout (salmo coruhensis). *Turkish Journal of Fisheries and Aquatic Sciences, 12*, 277-283. doi:10.4194/1303-2712-v12_2_12

Cao, G., Alessio, H. M., & Cutler, R. G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine, 14*(3), 303-311.

Chakravorty, S., Helb, D., Burday, M., Connell, N., & Alland, D. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria.*2, Journal of Microbiology*. Methods-330. doi:339

Chang, J., Shim, Y., Cha, S., & Chee, K. (2010). Probiotic characteristics of lactic acid bacteria isolated from kimchi. *Journal of Applied Microbiology, 109*(1), 220-230.

Chen, C., Chan, H. M., & Kubow, S. (2007). Kefir extracts suppress in vitro proliferation of estrogen-dependent human breast cancer cells but not normal mammary epithelial cells. *Journal of Medicinal Food, 10*(3), 416-422.

Chen, H. C., Wang, S. Y., & Chen, M. J. (2008). Microbiological study of lactic acid bacteria in kefir grains by culture-dependent and culture-independent methods. *Food Microbiology, 25*, 492-501. doi:10.1016/j.fm.2008.01.003

Chen, H. (2005). Boyden chamber assay. *Methods in Molecular Biology, 294*, 15-22.

Chen, Z., Shi, J., Yang, X., Nan, B., Liu, Y., & Wang, Z. (2014). Chemical and physical characteristics and antioxidant activities of the exopolysaccharide

produced by tibetan kefir grains during milk fermentation. *International Dairy Journal, 43*, 15-21. doi:10.1016/j.idairyj.2014.10.004

Chen, Z., Liang, K., Lin, Y., & Yang, F. (2013). Study of the UTMD-Based Delivery System to Induce Cervical Cancer Cell Apoptosis and Inhibit Proliferation with shRNA targeting Survivin. *International Journal of Molecular Sciences, 14*(1), 1763-1777. http://dx.doi.org/10.3390/ijms14011763

Clarridge, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiol Reviews, 4*, 840-862. doi:10.1128/CMR.17.4.840-862.2004

Colditz, G., & Dart, H. (2005). Cancer: Epidemiology and associations between diet and cancer. Encyclopedia of Human Nutrition, Second Edition.Elsevier, St.Louis, MO, 260-265.

Collins, J. A., Schandi, C. A., Young, K. K., Vesely, J., & Willingham, M. C. (1997). Major DNA fragmentation is a late event in apoptosis. *The Journal of Histochemistry and Cytochemistry : Official Journal of the Histochemistry Society, 45*(7), 923-934.

Coşkun, U., Günel, N., Sancak, B., Günel, U., Onuk, E., Bayram, O., . . . Ozkan, S. (2003). Significance of serum vascular endothelial growth factor, insulin-like growth factor-I levels and nitric oxide activity in breast cancer patients. *The Breast, 12*(2), 104-110.

Dalgleish, A. G., & Haefner, B. (2006). *THE LINK BETWEEN INFLAMMATION AND CANCER: Wounds that do not heal*. United States of America: Springer Science Business Media, Inc.

Das, D., & Goyal, A. (2005). Antioxidant activity and γ-aminobutyric acid (GABA) producing ability of probiotic lactobacillus plantarum DM5 isolated from marcha of sikkim. *LWT Food Science and Technology, 61*, 263-268. doi:10.1016/j.lwt.2014.11.013

De Luca, A., Lamura, L., Gallo, M., Maffia, V., & Normanno, N. (2012). Mesenchymal stem cell-derived interleukin-6 and vascular endothelial growth factor promote breast cancer cell migration. *Journal of Cellular Biochemistry, 113*(11), 3363-3370.

de Magalhães, J. P., Finch, C. E., & Janssens, G. (2010). Next-generation sequencing in aging research: Emerging applications, problems, pitfalls and possible solutions. *Ageing Research Reviews, 9*(3), 315-323.

de Moreno de LeBlanc, A., Matar, C., Farnworth, E., & Perdigon, G. (2006). Study of cytokines involved in the prevention of a murine experimental breast cancer by kefir. *Journal of Dairy Research, 90*, 1920-1928.

de Moreno de LeBlanc, A., Matar, C., Farnworth, E., & Perdigón, G. (2007). Study of immune cells involved in the antitumor effect of kefir in a murine breast cancer model. *Journal of Dairy Science, 90*, 1920-1928.

de Moreno de LeBlanc, A., Matar, C., LeBlanc, N., & Perdigón, G. (2005). Effects of milk fermented by *lactobacillus helveticus* R389 on a murine breast cancer model. *Breast Cancer Research, 7*, 477-486. doi:10.1186/bcr1032

De Vrese, M., Keller, B., & Barth, C. A. (1992). Enhancement of intestinal hydrolysis of lactose by microbial beta-galactosidase (EC 3.2.1.23) of kefir. *British Journal of Nutrition, 67*, 67-75.

DeNardo, D. G., & Coussens, L. M. (2007). Balancing immune response: Crosstalk between adaptive and innate immune cells during breast cancer progression. *Breast Cancer Research, 9*(4), 212.

Dent, R., Hanna, W. M., Trudeau, M., Rawlinson, E., Sun, P., & Narod, S. A. (2009). Pattern of metastatic spread in triple-negative breast cancer. *Breast Cancer Research and Treatment, 115*(2), 423-428.

Dent, R., Trudeau, M., Pritchard, K. I., Hanna, W. M., Kahn, H. K., Sawka, C. A., Lickley, L. A., Rawlinson, E., Narod, S. A. (2007). Triple-negative breast cancer: Clinical features and patterns of recurrence. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 13*(15 Pt 1), 4429-4434. doi:13/15/4429

Dimberg, A. (2010). Chemokines in angiogenesis. *The chemokine system in experimental and clinical hematology* (pp. 59-80) Springer.

Divisi, D., Di Tommaso, S., & Salvemini, S. (2006). Diet and cancer. *Acta Bio Medica Atenei Parmensis, 77*(2), 118-123.

Dobson, A., O'Sullivan, O., Cotter, P. D., Ross, P., & Hill, C. (2011). High-throughput sequence-based analysis of the bacterial composition of kefir and an associated kefir grain. *FEMS Microbiology Letters, 1*, 56-62. doi:10.1111/j.1574-6968.2011.02290

Eiro, N., & Vizoso, F. J. (2012). Inflammation and cancer. *World Journal of Gastrointestinal Surgery, 4*(3), 62-72. doi:10.4240/wjgs.v4.i3.62

Fantozzi, A., & Christofori, G. (2006). Mouse models of breast cancer metastasis. *Breast Cancer Research, 8*(4), 212.

Farnworth, E. R. (2006). Kefir–a complex probiotic. *Food Science and Technology Bulletin: Fu, 2*(1), 1-17.

Fernándes, L., & Steele, J. L. (1993). Glutathione content of lactic acid bacteria. *Journal of Dairy Science, 76*, 1233-1242. doi:10.3168/jds.S0022-0302(93)77452-4

Fidler, I. J. (2003). The pathogenesis of cancer metastasis: The'seed and soil'hypothesis revisited. *Nature Reviews Cancer, 3*(6), 453-458.

Florou-Paneri, P., Christaki, E., & Bonos, E. (2013). Lactic acid bacteria as source of functional ingredients. In M. Kongo (Ed.), *Lactic acid bacteria – R & D for*

food, health and livestock purposes (pp. 590-614) INTECH. doi:10.5772/47766

Folch-Cano, C., Jullian, C., Speisky, H., & Olea-Azar, C. (2010). Antioxidant activity of inclusion complexes of tea catechins with β-cyclodextrins by ORAC assays. *Food Research International, 8*, 2039-2044. doi:10.1016/j.foodres.2010.06.006

Folkman, J. (1972). Anti-angiogenesis: New concept for therapy of solid tumors. *Annals of Surgery, 175*(3), 409-416.

Foulkes, W. D., Smith, I. E., & Reis-Filho, J. S. (2010). Triple-negative breast cancer. *New England Journal of Medicine, 363*(20), 1938-1948.

Gao, J., Gu, F., He, J., Xiao, J., Chen, Q., Ruan, H., & He, G. (2013). Metagenome analysis of bacterial diversity in tibetan kefir grains. *Europian Food Research and Technology, 3*, 549-556. doi:10.1007/s00217-013-1912-2

Garcia-Etxebarria, K., Garcia-Garcerà, M., & Calafell, F. (2014). Consistency of metagenomic assignment programs in simulated and real data. *BMC Bioinformatics, 1*, 90.

Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. *Cell, 140*(6), 883-899.

Gu, L., Zhao, M., Li, W., You, L., Wang, J., Wang, H., & Ren, J. (2012). Chemical and cellular antioxidant activity of two novel peptides designed based on glutathione structure. *Food and Chemical Toxicology, 50*(11), 4085-4091.

Gülçin, İ. (2012). Antioxidant activity of food constituents: An overview. *Archives of Toxicology, 86*(3), 345-391.

Güven, A., & Gülmez, M. (2003). The effect of kefir on the activities of GSH-Px, GST, CAT, GSH and LPO levels in carbon Tetrachloride-Induced mice tissues. *Journal of Veterinary Medicine B, 50*, 412-416. doi:10.1046/j.1439-0450.2003.00693.x

Guzel-Seydim, Z., Wyffels, J. T., Seydim, A. C., & Greene, A. K. (2005). Turkish kefir and kefir grains: Microbial enumeration and electron microscobic observation†. *International Journal of Dairy Technology, 58*(1), 25-29.

Hamet, M. F., Londero, A., Medrano, M., Vercammen, E., Koenraad, V. H., Garrote, G. L., Huys, G., Vandamme, P., Abraham., A. G. (2013). Application of culture-dependent and culture-independent methods for the identification of lactobacillus kefiranofaciens in microbial consortia present in kefir grains. *Food Microbiology, 36*, 327-334. doi:10.1016/j.fm.2013.06.022

Hanahan, D., & Weinberg, R. A. (2011). Hallmark of cancer: The next generation. *Cell, 144*, 646-674. doi:10.1016/j.cell.2011.02.013

Hardy, H., Harris, J., Lyon, E., Beal, J., & Foey, A. D. (2013). Probiotics, prebiotics and immunomodulation of gut mucosal defences: Homeostasis and immunopathology. *Nutrients, 5*(6), 1869-1912.

Hart, A. L., Lammers, K., Brigidi, P., Vitali, B., Rizzello, F., Gionchetti, P., Campieri, M., Kamm, M. A., Knight, S. C., Stagg, A. J. (2004). Modulation of human dendritic cell phenotype and function by probiotic bacteria. *Gut, 53*(11), 1602-1609. doi:53/11/1602

Hasan, N. A., Young, B. A., Minard-Smith, A. T., Saeed, K., Li, H., Heizer, E. M., McMillan, J. N., Isom, R., Abdullah, S. A., Bornman, M. D., Faith, A. S., Choi, Y. S., Dickens, L. M., Cebula, A. T., Colwell, R. R. (2014). Microbial community profiling of human saliva using shotgun metagenomic sequencing. *PloS One, 5*, e97699.

Hicklin, D. J., & Ellis, L. M. (2005). Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 23*(5), 1011-1027. doi:JCO.2005.06.081 [pii]

Hirayama, K., & Rafter, J. (2000). The role of probiotic bacteria in cancer prevention. *Microbes and Infection, 2*(6), 681-686.

Hong, S., Bunge, J., Leslin, C., Jeon, S., & Epstein, S. S. (2009). Polymerase chain reaction primers miss half of rRNA microbial diversity. *The Isme Journal, 12*, 1365-1373. doi:10.1038/ismej.2009.89

Hoover, H. C., & Ketcham, A. S. (1975). Metastasis of metastases. *The American Journal of Surgery, 130*(4), 405-411.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricaltural and Food Chemistry., 50*, 4437-4444. doi:10.1021/jf0201529

Huang, Y., Wang, X., Wang, J., Wu, F., Sui, Y., Yang, L., & Wang, Z. (2013). Lactobacillus plantarum strains as potential probiotic cultures with cholesterol-lowering activity. *Journal of Dairy Research, 96*, 2816-2825.

Hui, Y. H., & Evranuz, E. Ö. (2012). *Handbook of animal-based fermented food and beverage technology* CRC Press.

Huseini, H. F., Rahimzadeh, G., Fazeli, M. R., Mehrazma, M., & Salehi, M. (2012). Evaluation of wound healing activities of kefir products. *Burns, 38*, 719-723.

Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2007a). MEGAN analysis of metagenomic data. *Genome Research, 17*(3), 377-386. doi:gr.5969107 [pii]

Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2007b). MEGAN analysis of metagenomic data. *Genome Research, 17*(3), 377-386. doi:gr.5969107 [pii]

Illumina Sign In. (2015). *Basespace.illumina.com*. Retrieved 20 November 2015, from http://basespace.illumina.com/apps/593593/16S-Metagenomics

Inoue, M., Suzuki, R., Koide, T., Sakaguchi, N., Ogihara, Y., & Yabu, Y. (1994). Antioxidant, gallic acid, induces apoptosis in HL-60RG cells. . *Biochemical and Biophysical Research Communications, 204*, 898-804. doi:10.1006/bbrc.1994.2544

Isolauri, E., Sutas, Y., Kankaanpaa, P., Arvilommi, H., & Salminen, S. (2001). Probiotics: Effects on immunity. *The American Journal of Clinical Nutrition, 73*(2 Suppl), 444S-450S.

Jaiswal, M., LaRusso, N. F., Burgart, L. J., & Gores, G. J. (2000). Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Research, 60*(1), 184-190.

Jarnicki, A. G., Lysaght, J., Todryk, S., & Mills, K. H. (2006). Suppression of antitumor immunity by IL-10 and TGF-beta-producing T cells infiltrating the growing tumor: Influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells. *Journal of Immunology (Baltimore, Md.: 1950), 177*(2), 896-904. doi:177/2/896

Jung, C., Hugot, J. P., & Barreau, F. (2010). Peyer's patches: The immune sensors of the intestine. *International Journal of Inflammation, 2010*, 823710. doi:10.4061/2010/823710

Kacinski, B. M. (1995). CSF-1 and its receptor in ovarian, endometrial and breast cancer. *Annals of Medicine, 27*(1), 79-85.

Kakisu, E., Irigoyen, A., Torre, P., De Antoni, G. L., & Abraham, A. G. (2011). Physicochemical, microbiological and sensory profiles of fermented milk containing probiotic strains isolated from kefir. *Journal of Dairy Research, 78*, 456-463. doi:doi: 10.1017/S0022029911000653

Kakiuchi-Kiyota, S., Lappin, P. B., Heintz, C., Brown, P. W., Pinho, F. O., Ryan, A. M., & Mathialagan, N. (2014). Expression of proto-oncogene cFMS protein in lung, breast, and ovarian cancers. *Applied Immunohistochemistry & Molecular Morphology : AIMM / Official Publication of the Society for Applied Immunohistochemistry, 22*(3), 188-199. doi:10.1097/PAI.0b013e31828e7104

Kameya, H., Watanabe, J., Takano-Ishikawa, Y., & Todoriki, S. (2014). Comparison of scavenging capacities of vegetables by ORAC and EPR. *Food Chemistry, 145*, 866-873. doi:10.1016/j.foodchem.2013.09.015

Kaur, P., Nagaraja, G. M., Zheng, H., Gizachew, D., Galukande, M., Krishnan, S., & Asea, A. (2012). A mouse model for triple-negative breast cancer tumor-initiating cells (TNBC-TICs) exhibits similar aggressive phenotype to the human disease. *BMC Cancer, 12*, 120-2407-12-120. doi:10.1186/1471-2407-12-120

Kedare, S. B., & Singh, R. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology, 48*(4), 412-422.

Keller, P. J., Arendt, L. M., Skibinski, A., Logvinenko, T., Klebba, I., Dong, S., Smith, A. E., Prat, A., Perou, C. M., Gilmore, H., Schnitt, S., Naber, S. P., Garlick, J. A., Kuperwasser, C. (2012). Defining the cellular precursors to human breast cancer. *Proceedings of the National Academy of Sciences of the United States of America, 109*(8), 2772-2777. doi:10.1073/pnas.1017626108

Kesmen, Z., & Kacmaz, N. (2011). Determination of lactic microflora of kefir grains and kefir beverage by using Culture-Dependent and Culture-Independent methods. *Journal of Food Science, 76*, M276-M283. doi:10.1111/j.1750-3841.2011.02191.x

Kilic, N., Taslipinar, M. Y., Guney, Y., Tekin, E., & Onuk, E. (2014). An investigation into the serum thioredoxin, superoxide dismutase, malondialdehyde, and advanced oxidation protein products in patients with breast cancer. *Annals of Surgical Oncology, 21*(13), 4139-4143.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2012). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 1*, gks808. doi:10.1093/nar/gks808

Knutson, K., & Disis, M. (2005). Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. *Cancer Immunology, Immunotherapy, 54*(8), 721-728.

Kodali, V. P., & Sen, R. (2008). Antioxidant and free radical scavenging activities of an exopolysaccharide from a probiotic bacterium. *Biotechnology Journal., 3*, 245-251. doi:10.1002/biot.200700208

Konczak, I., Zabaras, D., Dunstan, M., & Aguas, P. (2010). Antioxidant capacity and phenolic compounds in commercially grown native Australian herbs and spices. *Food Chemistry, 122*, 260-266. doi:10.1016/j.foodchem.2010.03.004

Koopman, G., Reutelingsperger, C., Kuijten, G., Keehnen, R., Pals, S., & Van Oers, M. (1994). Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood, 84*(5), 1415-1420.

Korkaya, H., Lee, E., Ouzounova, M., El Andaloussi, A., Novakovic, E., Piranlioglu, R., Demirci, M. F., Clouthier, S. G., Wicha, M. S. (2015). Systemic early immune priming via tumor-secreted cytokines facilitates breast cancer metastasis in syngeneic mouse model. *Cancer Research, 75*(15 Supplement), 466-466.

Kozyrovska, N., Reva, O., & Goginyan, V. (2012). Kombucha microbiome as a probiotic: A view from the perspective of post-genomics and synthetic ecology. *Biopolymers and Cell*, (28,№ 2), 103-113.

Kudo, Y., Iizuka, S., Yoshida, M., Tsunematsu, T., Kondo, T., Subarnbhesaj, A., deraz, E. M., Tahara, H., Ishimaru, N., Ogawa, I., Takata, T. (2012). Matrix

metalloproteinase-13 (MMP-13) directly and indirectly promotes tumor angiogenesis. *The Journal of Biological Chemistry, 287*(46), 38716-38728. doi:10.1074/jbc.M112.373159

Kumar, M., Kumar, A., Nagpal, R., Mohania, D., Behare, P., Verma, V., Kumar, P., Poddar, D., Aggarwal, P. K., Henry, C., Jain, S., Yadav, H. (2010). Cancer-preventing attributes of probiotics: An update. *International Journal of Food Sciences and Nutrition, 61*(5), 473-496.

Lala, P. K., & Chakraborty, C. (2001). Role of nitric oxide in carcinogenesis and tumour progression. *The Lancet Oncology, 2*(3), 149-156.

Le Leu, R. K., Brown, I. L., Hu, Y., Bird, A. R., Jackson, M., Esterman, A., & Young, G. P. (2005). A synbiotic combination of resistant starch and bifidobacterium lactis facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. *Journal of Nutrition, 135*, 996-1001.

Leber, M. F., & Efferth, T. (2009). Molecular principles of cancer invasion and metastasis (review). *International Journal of Oncology, 34*(4), 881-895.

Lee, H. S., Ha, A. W., & Kim, W. K. (2012). Effect of resveratrol on the metastasis of 4T1 mouse breast cancer cells in vitro and in vivo. *Nutrition Research and Practice, 6*(4), 294-300.

Lee, J. W., Kim, E. H., Yim, I. B., & Joo, H. G. (2004). Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of lactobacillus casei and bifidobacterium longum. *Journal of Veterinary Science, 5*(1), 41-48.

Lee, M. Y., Ahn, K. S., Kwon, O. K., Kim, M. J., Lee, I. Y., Oh, S. R., & Lee, H. K. (2007). Anti-inflammatory and anti-allergenic effects of kefir in mouse asthma model. *Immunobiology, 212*, 647-654.

Leibovich-Rivkin, T., Liubomirski, Y., Bernstein, B., Meshel, T., & Ben-Baruch, A. (2013). Inflammatory factors of the tumor microenvironment induce plasticity in nontransformed breast epithelial cells: EMT, invasion, and collapse of normally organized breast textures. *Neoplasia, 15*(12), 1330-IN5.

Leite, A. M. O., Mayo, B., Rachid, C. T. C. C., Peixoto, R. S., Silva, J. T., Paschoalin, V. M. F., & Delgado, S. (2012). Assessment of the microbial diversity of brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. *Food Microbiology, 31*, 215-221. doi:10.1016/j.fm.2012.03.011

Liang, C. C., Park, A. Y., & Guan, J. L. (2007). *In vitro* scratch assay: A convenient and inexpensive method for analysis of cell migration *in vitro*. *Nature Protocol, 2*, 329-333. doi:10.1038/nprot.2007.30

Liu, J. R., Chen, M. J., & Lin, C. W. (2005). Antimutagenic and antioxidant properties of milk-kefir and soymilk-kefir. *Journal of Agricultural and Food Chemistry, 53*, 2467-2474. doi:10.1021/jf048934k

Liu, J. R., Lin, Y. Y., Chen, M. J., Chen, L. J., & Lin, C. W. (2005). Antioxidative activities of kefir. *Asian-Australasian Journal of Animal Sciences, 18*, 567-573.

Liu, J., Wang, S., Lin, Y., & Lin, C. (2002). Antitumor activity of milk kefir and soy milk kefir in tumor-bearing mice. *Nutrition and Cancer, 44*(2), 183-187.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods, 25*(4), 402-408.

Locatelli, C., Filippin-Monteiro, F. B., & Creczynski-Pasa, T. B. (2013). Alkyl esters of gallic acid as anticancer agents: A review. *European Journal of Medicinal Chemistry, 60*, 233-239.

Mahmood, T., & Yang, P. (2012). Western blot: Technique, theory, and trouble shooting. *North American Journal of Medical Sciences, 4*(9), 429.

Makwana, R., Pawar, M., Chauhan, H., & Bhagwat, S. (2014). Role of probiotic in milk and its byproducts. *Journal of Animal Feed Science and Technology, 2*(2), 91.

Manzari, C., Fosso, B., Marzano, M., Annese, A., Caprioli, R., D'Erchia, A. M., Gissi, C., Intranuovo, M., Picardi, E., Santamaria, M., Scorrano, S., Sgaramella, G., Pesole, G. (2014). The influence of invasive jellyfish blooms on the aquatic microbiome in a coastal lagoon (varano, SE italy) detected by an illumina-based deep sequencing strategy. *Biological Invasions, 3*, 923-940.

Martin, T. A., Ye, L., Sanders, A. J., Lane, J., & Jiang, W. G. (2000). Cancer invasion and metastasis: Molecular and cellular perspective.

Masaaki, K., Yongkun, S., Miyako, K., Miki, M., Toshiyuki, H., & Takeshi, S. (2012). Measurement of DNA damage by terminal deoxynucleotidyl transferase reaction. *Advances in Biological Chemistry, 2012*

Masson, V., Devy, L., Grignet-Debrus, C., Bernt, S., Bajou, K., Blacher, S., Roland, G., Chang, Y., Fong, T., Carmeliet, P., Foidart, J., Noël, A. (2002). Mouse aortic ring assay: A new approach of the molecular genetics of angiogenesis. *Biological Procedures Online, 4*(1), 24-31.

Mehner, C., Hockla, A., Miller, E., Ran, S., Radisky, D. C., & Radisky, E. S. (2014). Tumor cell-produced matrix metalloproteinase 9 (MMP-9) drives malignant progression and metastasis of basal-like triple negative breast cancer. *Oncotarget, 5*(9), 2736-2749.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunology Methods, 65*(1-2), 55-63.

Murofushi, M., Mizuguchi, J., & Albara, K. (1986). Immunopotentiative effect of polysaccharide from kefir grain, KGF-C, administered orally in mice. *Immunopharmacol, 12*, 29-35.

Murooka, Y., & Yamshita, M. (2008). Traditional healthful fermented products of japan. *Journal of Industrial Microbiology & Biotechnology, 35*(8), 791-798.

Najgebauer-Lejko, D. (2014). Effect of green tea supplementation on the microbiological, antioxidant, and sensory properties of probiotic milks. *Dairy Science and Technology, 94*, 327-339. doi:10.1007/s13594-014-0165-6

Nalbantoglu, U., Cakar, A., Dogan, H., Abaci, N., Ustek, D., Sayood, K., & Can, H. (2014). Metagenomic analysis of the microbial community in kefir grains. *Food Microbiology, 41*, 42-51. doi:10.1016/j.fm.2014.01.014

Nielsen, B., Gürakan, G. C., & Ünlü, G. (2014). Kefir: A multifaceted fermented dairy product. *Probiotics and Antimicrobial Proteins, 3-4*, 123-135. doi:10.1007/s12602-014-9168-0

Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions–a review. *International Journal of Medical Microbiology, 300*(1), 57-62.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351-358.

Omar, Z. A., Mohd.Ali, Z., & Ibrahim Tamin, N. S. (2006). *Malaysian cancer statistics– data and figure peninsular Malaysia 2006*.

Ot1es, S., & Cagindi, O. (2003). Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. *Pakistan Journal of Nutrition., 2*, 54-59.

Otles, S., Cagindi, O., & Akcicek, E. (2003). Probiotics and health. *Asian Pacific Journal of Cancer Prevention., 4*, 369-372.

Parham, P. (2014). *The immune system.* Garland Science.

Parvez, S., Malik, K., Ah Kang, S., & Kim, H. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology, 100*(6), 1171-1185.

Persichetti, E., De Michele, A., Codini, M., & Traina, G. (2014). Antioxidative capacity of lactobacillus fermentum LF31 evaluated in vitro by oxygen radical absorbance capacity assay. *Nutrition, 30*, 936-938. doi:10.1016/j.nut.2013.12.009

Petrella, B. L., Armstrong, D. A., & Vincenti, M. P. (2012). Interleukin-1 beta and transforming growth factor-beta 3 cooperate to activate matrix metalloproteinase expression and invasiveness in A549 lung adenocarcinoma cells. *Cancer Letters, 325*(2), 220-226.

Piccolo, E., Tinari, N., Semeraro, D., Traini, S., Fichera, I., Cumashi, A., Sorda, R. L., Spinella, F., Bagnato, A., Lattanzio, R., D'Egidio, M., Risio, A. D., Stampolidis, P., Piantelli, M., Natoli, C., Ullrich, A., Lacobelli, S. (2013). LGALS3BP, lectin galactoside-binding soluble 3 binding protein, induces vascular endothelial growth factor in human breast cancer cells and promotes angiogenesis. *Journal of Molecular Medicine, 91*(1), 83-94.

Pulaski, B. A., & Ostrand-Rosenberg, S. (1998). Reduction of established spontaneous mammary carcinoma metastases following immunotherapy with major histocompatibility complex class II and B7.1 cell-based tumor vaccines. *Cancer Research, 58*, 1486-1493.

Pulaski, B. A., & Ostrand-Rosenberg, S. (2001). Mouse 4T1 breast tumor model. *Current Protocols in Immunology,* , 20.2. 1-20.2. 16.

Pulaski, B. A., & Ostrand-Rosenberg, S. (1998). Reduction of established spontaneous mammary carcinoma metastases following immunotherapy with major histocompatibility complex class II and B7.1 cell-based tumor vaccines. *Cancer Research, 58*(7), 1486-1493.

Rakoff-Nahoum, S. (2006). Why cancer and inflammation? *The Yale Journal Biology and Medicine, 79*, 123-130.

Redelman, D., & Hunter, K. W. (2007). The mouse mammary carcinoma 4T1: Characterization of the cellular landscape of primary tumours and metastatic tumour foci. *International Journal of Experimental Pathology, 88*(5), 351-360.

Rodrigues, K. L., Carvalho, J. C., & Schneedorf, J. M. (2005). Anti-inflammatory properties of kefir and its polysaccharide extract. *Inflammopharmacology, 13*, 485-492.

Rodríguez, H., Curiel, J. A., Landete, J. M., de las Rivas, B., de Felipe, F. L., Gómez-Cordovés, C., Mancheno, J. M., Muñoz, R. (2009). Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology, 132*, 79-90. doi:10.1016/j.ijfoodmicro.2009.03.025

Rolfe, R. D. (2000). The role of probiotic cultures in the control of gastrointestinal health. *The Journal of Nutrition, 130*(2S Suppl), 396S-402S.

Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts torilisleptophylla L. *BMC Complementary and Alternative Medicine, 1*, 221.

Saide, J. A. O., & Gilliland, S. E. (2005). Antioxidative activity of lactobacilli measured by oxygen radical absorbance capacity. *Journal of Dairy Science, 88*, 1352-1357. doi:10.3168/jds.S0022-0302(05)72801-0

Samarajeewa, A., Hammad, A., Masson, L., Khan, I., Scroggins, R., & Beaudette, L. (2015). Comparative assessment of next-generation sequencing, denaturing gradient gel electrophoresis, clonal restriction fragment length polymorphism and cloning-sequencing as methods for characterizing commercial microbial consortia. *Journal of Microbiological Methods, 108*, 103-111.

Samarghandian, S., & Shabestari, M. M. (2013). DNA fragmentation and apoptosis induced by safranal in human prostate cancer cell line. *Indian Journal of Urology: IJU: Journal of the Urological Society of India, 29*(3), 177-183. doi:10.4103/0970-1591.117278

Sanders, M. E., Guarner, F., Guerrant, R., Holt, P. R., Quigley, E. M., Sartor, R. B., Sherman, P, M., Mayer, E. A. (2013). An update on the use and investigation of probiotics in health and disease. *Gut, 62*(5), 787-796. doi:10.1136/gutjnl-2012-302504

Sapi, E., & Kacinski, B. M. (1999). The role of CSF-1 in normal and neoplastic breast physiology. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.), 220*(1), 1-8.

Satir, G., & Guzel-Seydim, Z. B. (2015). Influence of kefir fermentation on the bioactive substances of different breed goat milks. *LWT-Food Science and Technology,*

Schiffrin, E., Rochat, F., Link-Amster, H., Aeschlimann, J., & Donnet-Hughes, A. (1995). Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *Journal of Dairy Science, 78*(3), 491-497.

Scholl, S. M., Pallud, C., Beuvon, F., Hacene, K., Stanley, E. R., Rohrschneider, L., Tang, R., Pouillart, P., Lidereau, R. (1994). Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. *Journal of the National Cancer Institute, 86*(2), 120-126.

Schreibelt, G., Bol, K. F., Aarntzen, E. H. J. G., Gerritsen, W. R., Punt, C. J. A., Figdor, C. G., & de Vries, I. J. M. (2013). Importance of helper T-cell activation in dendritic cell-based anticancer immunotherapy. *Oncoimmunology, 2*(6), e24440. doi:10.4161/onci.24440

Scully, O. J., Bay, B. H., Yip, G., & Yu, Y. (2012). Breast cancer metastasis. *Cancer Genomics and Proteomics, 9*, 311-320.

Shan, D., Chen, L., Njardarson, J. T., Gaul, C., Ma, X., Danishefsky, S. J., & Huang, X. Y. (2005). Synthetic analogues of migrastatin that inhibit mammary tumor metastasis in mice. *Proceedings of the National Academy of Sciences of the United States of America, 102*(10), 3772-3776. doi:0500658102

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry, 113*(4), 1202-1205.

Sharma, R., Kapila, R., Kapasiya, M., Saliganti, V., Dass, G., & Kapila, S. (2014). Dietary supplementation of milk fermented with probiotic lactobacillus fermentum enhances systemic immune response and antioxidant capacity in aging mice. *Nutrition Research, 34*, 968-981. doi:10.1016/j.nutres.2014.09.006

Sharma, S., Stolina, M., Lin, Y., Gardner, B., Miller, P. W., Kronenberg, M., & Dubinett, S. M. (1999). T cell-derived IL-10 promotes lung cancer growth by suppressing both T cell and APC function. *Journal of Immunology (Baltimore, Md.: 1950), 163*(9), 5020-5028. doi:ji_v163n9p5020

Shiomi, M., Sasaki, K., Murofushi, M., & Albara, K. (1982). Antitumor activity in mice of orally administered polysaccharide from kefir grain. *Journal of Medical Science, 35*, 75-80.

Siege, R., Ma, J., Zou, Z., & Jemal, A. (2014). Cancer statistics, 2014. *CA Cancer Journal of Clinicians, 64*, 9-29. doi:10.3322/caac.21208

Simova, E., Beshkova, D., Angelov, A., Hristozova, T., Frengova, G., & Spasov, Z. (2002). Lactic acid bacteria and yeasts in kefir grains and kefir made from them. *Journal of Industrial Microbiology and Biotechnology, 28*, 1-6. doi:10.1038/sj.jim.7000186

Sredni, B., Weil, M., Khomenok, G., Lebenthal, I., Teitz, S., Mardor, Y., Ram, Z., Orenstein, A., Kershenovich, A., Michowiz, S., Cohen, Y. I., Rappaport, Z. H., Freidkin, I., Albeck, M., Longo, D., Kalechman, Y. (2004). Ammonium trichloro(dioxoethylene-o,o')tellurate (AS101) sensitizes tumors to chemotherapy by inhibiting the tumor interleukin 10 autocrine loop. *Cancer Research, 64*, 1843-1852.

Stanley, E. R., Guilbert, L. J., Tushinski, R. J., & Bartelmez, S. (1983). CSF-1—A mononuclear phagocyte lineage-specific hemopoietic growth factor. *Journal of Cellular Biochemistry, 21*(2), 151-159.

Sugden, D., & de Winter, P. (2008). Quantification of mRNA using real time RT-PCR. *Molecular biomethods handbook* (pp. 149-168) Springer.

Sun, Y. P., Chou, C. C., & Yu, R. C. (2009). Antioxidant activity of lactic-fermented chinese cabbage. *Food Chemistry, 115*, 912-917. doi:10.1016/j.foodchem.2008.12.097

Takizawa, S., Kojima, S., Tamura, S., Fujinaga, S., Benno, Y., & Nakase, T. (1998). The composition of the lactobacillus flora in kefir grains. *Systematic and Applied Microbiology, 21*, 121-127. doi:10.1016/S0723-2020(98)80015-5

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. . *Journal of Food Composition and Analysis, 6*, 669-675. doi:10.1016/j.jfca.2006.01.003

Timofte, D., Eva, L., Vasincu, D., Buzea, C. G., Agop, M., & Popa, R. F. (2015). Implications of the "Subquantum level" in carcinogenesis and tumor progression via scale relativity theory.

Valentiner, U., Brooks, S. A., & Schumacher, U. (2006). In vivo xenograft models of breast cancer metastasis. *Breast cancer research protocols* (pp. 479-488) Springer.

van Zijl, F., Krupitza, G., & Mikulits, W. (2011). Initial steps of metastasis: Cell invasion and endothelial transmigration. *Mutation Research/Reviews in Mutation Research, 728*(1), 23-34.

Van't Veer, L. J., Dai, H., Van De Vijver, Marc J, He, Y. D., Hart, A. A., Mao, M., Peterse, H. L., Van Der Kooy, K., Marton, M. J., Witteveen, A. T., Schreiber, G., J., Kerkhoven, R. M., Roberts, C., Linsley, P. S., Bernards, R., Witteveen, A. T. (2002). Gene expression profiling predicts clinical outcome of breast cancer. *Nature, 415*(6871), 530-536.

Vardjan, T., Mohar Lorbeg, P., Rogelj, I., & Čanžek Majhenič, A. (2013). Characterization and stability of lactobacilli and yeast microbiota in kefir grains. *Journal of Dairy Research, 5*, 2729-2736. doi:10.3168/jds.2012-5829

Vinderola, C. G., Duarte, J., Thangavel, D., Perdigón, G., Farnworth, E., & Matar, C. (2005a). Immunomodulating capacity of kefir. *Journal of Dairy Research, 72*, 195-202.

Vinderola, C. G., Duarte, J., Thangavel, D., Perdigón, G., Farnworth, E., & Matar, C. (2005b). Immunomodulating capacity of kefir. *Journal of Dairy Research, 72*(02), 195-202.

Voronov, E., Shouval, D. S., Krelin, Y., Cagnano, E., Benharroch, D., Iwakura, Y., Dinarello, C. A., Apte, R. N. (2003). IL-1 is required for tumor invasiveness and angiogenesis. *Proceedings of the National Academy of Sciences of the United States of America, 100*(5), 2645-2650. doi:10.1073/pnas.0437939100

Wan, L., Pantel, K., & Kang, Y. (2013). Tumor metastasis: Moving new biological insights into the clinic. *Nature Medicine, 19*(11), 1450-1464.

Wang, S. Y., Chen, K. N., Lo, Y. M., Chiang, M. L., Chen, H. C., Liu, J. R., & Chen, M. J. (2012). Investigation of microorganisms involved in biosynthesis of the kefir grain. *Food Microbiology, 32*, 274-285. doi:10.1016/j.fm.2012.07.001

Wang, Y. Y., Li, H. R., Jia, S. F., Wu, Z. J., & Guo, B. H. (2006). Analysis of bacterial diversity of kefir grains by denaturing gradient gel electrophoresis and 16S rDNA sequencing.*46*, 310-313.

Wang, Y., Xu, N., Xi, A., Ahmed, Z., Zhang, B., & Bai, X. (2009). Effects of lactobacillus plantarum MA2 isolated from tibet kefir on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. *Applied Microbiology and Biotechnology, 84*, 341-347.

Wang, Z., Huang, C., Li, Z., Yang, J., Li, B., Liang, R., Dai, Z., Liu, Z. (2010). Chrysanthemum indicum ethanolic extract inhibits invasion of hepatocellular carcinoma via regulation of MMP/TIMP balance as therapeutic target. *Oncology Reports, 23*(2), 413-421.

Whiteside, T. L. (2006). Immune suppression in cancer: Effects on immune cells, mechanisms and future therapeutic intervention. *Seminars in Cancer Biology., 16*(1) 3-15.

Workman, P., Aboagye, E., Balkwill, F., Balmain, A., Bruder, G., Chaplin, D., Double, J. A., Everitt, J., Farningham, D. A. H., Glennie, M., Kelland, L. R., Robinson, V., Stratford, I. J., Tozer, G. M., Watson, S., Wedge, S. R., Eccles, S. A.

(2010). Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer, 102*(11), 1555-1577.

World Health Organization. (2006). *Probiotics in food: Health and nutritional properties and guidelines for evaluation* Food and Agriculture Organization of the United Nations.

Yeap, S. K., Yusof, H., Mohamad, N. E., Boon, K. B., Ho, W. Y., Ali, N. M., Alitheen, N. J., Koh, S. P., Long, K. (2013). In vivo immunomodulation and lipid peroxidation activities contributed to chemoprevention effects of fermented mung bean against breast cancer. *Evidence-Based Complementary and Alternative Medicine, 2013*, 1-7. doi:http://dx.doi.org/10.1155/2013/708464

Yıldırım, İ. H., Yıldırım, S. C., & Koçak, N. (2011). Molecular methods for bacterial genotyping and analyzed gene regions. *Journal of Microbiology and Infection Diseases,* 1, 42-46. doi:10.5799/ahinjs.02.2011.01.0011

Yip, C. H., Taib, N., & Mohamed, I. (2006). Epidemiology of breast cancer in malaysia. *Asian Pacific Journal of Cancer Prevention,* 7(3), 369.

Zanirati, D. F., Abatemarco, M., de CiccoSandes, S. H., Nicoli, J. R., Nunes, Á. C., & Neumann, E. (2014). Selection of lactic acid bacteria from brazilian kefir grains for potential use as starter or probiotic cultures. *Anaerobe, 32*, 70-76. doi:10.1016/j.anaerobe.2014.12.007

Zetter, P., Bruce R. (1998). Angiogenesis and tumor metastasis. *Annual Review of Medicine, 49*(1), 407-424.

Zulueta, A., Esteve, M. J., & Frígola, A. (2009). ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry, 114*(1), 310-316.

BIODATA OF STUDENT

The student, Nur Rizi Binti Zamberi, was born in Ipoh, Perak on 1st September 1990 and was raised in Ipoh since then. She went through her primary education in Sekolah Kebangsaan Seri Ampang, Ipoh and later attended her lower secondary education in Sekolah Kebangsaan Wira Jaya, Ipoh. After PMR, she finished her secondary education in Maktab Rendah Sains Mara Balik Pulau, Pulau Pinang. Then, she received a MARA scholarship to pursue her foundation in American Degree Foundation Program in INTEC, UiTM Shah Alam and then her Bachelor degree in Rochester Institute of Technology, New York, USA for four years. She received her Bachelor of Science in Biomolecular Science and Biotechnology in May 2013. On the same year, she started her Master degree research in Animal Tissue Culture Lab (ATCL) at Faculty of Biotechnology and Biomolecular Science.

LIST OF PUBLICATIONS

Zamberi, N., Abu, N., Mohamed, N., Nordin, N., Keong, Y., & Beh, B. et al. (2016). The Antimetastatic and Antiangiogenesis Effects of Kefir Water on Murine Breast Cancer Cells. *Integrative Cancer Therapies*. http://dx.doi.org/10.1177/1534735416642862

Zamberi, N., Mohamad, N., Yeap, S. K., Ky, H., Beh, B. K., Liew, W. C., Tan, S. W., Ho, W. Y., Boo, S. Y., Chua, Y. H., Alitheen, N. B. (2016). 16S Metagenomic: microbial composition analysis of kefir grain using MEGAN and BaseSpace. *Food Biotechnology.* (Accepted)

Abu, N., Yeap, S., Pauzi, A., Akhtar, M., Zamberi, N., & Ismail, J. et al. (2016). Dual Regulation of Cell Death and Cell Survival upon Induction of Cellular Stress by Isopimara-7,15-Dien-19-Oic Acid in Cervical Cancer, HeLa Cells In vitro. *Frontiers In Pharmacology, 7*. http://dx.doi.org/10.3389/fphar.2016.00089

Nordin, N., Yeap, S., Abu, N., Hashim, M., Zamberi, N., & Alitheen, N. et al. (2016). Characterisation of sol–gel method synthesised MgZnFe2O4 nanoparticles and its cytotoxic effects on breast cancer cell line, MDA MB-231 in vitro. *IET Nanobiotechnology*. http://dx.doi.org/10.1049/iet-nbt.2016.0007

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

MICROBIOTA PROFILE OF MALAYSIAN KEFIR GRAIN AND ANTI-METASTATIC EFFECTS OF KEFIR WATER IN MURINE BREAST CANCER CELLS

NAME OF STUDENT: NUR RIZI ZAMBERI

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from_____ until _____ (date) (date)

Approved by:

_____	_____
(Signature of Student)	(Signature of Chairman of Supervisory Committee)
New IC No/ Passport No.:	Name:
Date :	Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]