

**EFFECTS OF A LACTOBACILLI PROBIOTIC
ON SURVIVAL AND MOBILITY OF
FEMALE *DROSOPHILA MELANOGASTER*
WITH ALZHEIMER'S DISEASE**

LIM HUI TING

UNIVERSITI SAINS MALAYSIA

JUNE 2020



**PUSAT PENGAJIAN TEKNOLOGI INDUSTRI
UNIVERSITI SAINS MALAYSIA**

**BORANG PENYERAHAN DISERTAI MUTAKHIR
SATU (1) NASKAH**

Nama Penyelia: PROF. DR. LIONG MIN-TZE

Bahagian: TEKNOLOGI BIOPROSES

Saya telah menyemak semua pembetulan/pindaan yang dilaksanakan oleh
~~Encik/Puan~~/Cik LIM HUI TING

mengenai disertainya sebagaimana yang dipersetujui oleh Panel Pemeriksa di Viva Vocenya.

2. Saya ingin mengesahkan bahawa saya berpuashati dengan pembetulan/pindaan yang dilaksanakan oleh calon.

Sekian, terima kasih.


PROF. DR. LIONG MIN TZE
Ph.D. (Melb) Australia
School of Industrial Technology
Universiti Sains Malaysia. (USM)
11800 Penang, Malaysia

Tandatangan dan cop

17 JULAI 2020

Tarikh



**EFFECTS OF A LACTOBACILLI PROBIOTIC
ON SURVIVAL AND MOBILITY OF
FEMALE *DROSOPHILA MELANOGASTER*
WITH ALZHEIMER'S DISEASE**

by

LIM HUI TING

A dissertation submitted in the partial fulfillment of the requirements for the degree of
Bachelor of Technology (B.Tech) in the field of Bioprocess Technology
School of Industrial Technology
Universiti Sains Malaysia

June 2020

DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institutions.

A handwritten signature in cursive script that reads "Athena".

LIM HUI TING

JUNE 2020

ACKNOWLEDGEMENTS

I wish to express my heartiest gratitude to my supervisor, Prof. Dr. Liong Min-Tze, for providing me an opportunity to use *Drosophila melanogaster* as animal models for human Alzheimer's disease during my final year project. My appreciation to her for extended much understanding, wisdom, and patience in providing valuable comments and feedbacks at various stages of this research project.

Besides, I would like to thank my co-supervisor, Dr. Mohd Ghows Mohd Azzam from the School of Biological Sciences, Universiti Sains Malaysia, for allowing me to have access to the facilities in molecular biology lab to carry out my final year project much to my convenience, and his postgraduate student, Mr. Andrew Chung Jie Ting for willing to share his expertise by teaching me on the operation of *Toxtrac* software.

I am extremely grateful to the research officers, Mr. Ahmad Imran Zaydi, Ms. Fiona Chung Yi Li and Mr. Lee Boon Kiat for their kind guidance and assistance with their experiences in solving problems and perspectives on the research topic. Last but not least, I would like to express my cordial thanks to the final year project students from School of Biological Sciences, for willing to share knowledge with me on *Drosophila* maintenance, in which I really appreciate for their encouragement, motivation and support throughout my final year project.

Lim Hui Ting

June 2020

TABLE OF CONTENTS

	Page
Acknowledgements	iii
Table of Contents	iv
List of Tables	vi
List of Figures	vii
List of Symbols and Abbreviations	x
Abstrak	xii
Abstract	xiv
CHAPTER 1 INTRODUCTION	
1.1 Research background	1
1.2 Problem statement	3
1.3 Research scope and objectives	5
CHAPTER 2 LITERATURE REVIEW	
2.1 A β 42 peptide as biomarker for Alzheimer's disease	7
2.2 The relation between aging and Alzheimer's disease	9
2.3 <i>Drosophila melanogaster</i> as potential model for aging-associated Alzheimer's disease	10
2.4 UAS/GAL4 expression system as genetic tools for <i>Drosophila melanogaster</i>	13
2.5 Pathological effects of human A β 42 peptide in <i>Drosophila melanogaster</i>	15
2.6 Health-promoting benefits of probiotic	16

CHAPTER 3 MATERIALS AND METHODS	
3.1 Bacterial strains and media	19
3.2 <i>Drosophila</i> stocks and maintenance	19
3.3 Survival analysis	21
3.4 Mobility analysis	22
3.5 Statistical analysis	25
CHAPTER 4 RESULTS AND DISCUSSION	
4.1 Survival	26
4.2 Mobility	30
4.2.1 Percentage of flies that crossed half-height of the vial in 5 seconds	30
4.2.2 Speed for climbing to the top	35
4.2.3 Frequency of flies falling	39
4.2.4 Total distance travelled to the top	42
4.2.5 Negative geotactic index	45
CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS	
5.1 Conclusion	48
5.2 Recommendations for future research	49
REFERENCES	50
APPENDICES	59

LIST OF TABLES

Table Caption	Page
3.1 List of <i>Drosophila melanogaster</i> strains with description.	20
3.2 List of <i>Drosophila</i> lines and strain of <i>Lactobacillus</i> used.	21

LIST OF FIGURES

Figure Caption	Page
2.1 The bipartite UAS/GAL4 expression system in <i>Drosophila melanogaster</i> , where males carrying a GAL4 driver under the control of Act5C promoter were mated to virgin females carrying upstream activation sequences (UAS) with downstream target gene, amyloid beta (A β) 42. Female progenies containing both elements of the system were collected for this project as <i>Drosophila</i> Alzheimer's disease (AD) model. In the progeny of the cross, the Act5C promoter in GAL4 driver drives the expression of GAL4 yeast transcription factor in <i>Drosophila</i> ubiquitously, while GAL4 will specifically binds to multiple binding sites in yeast galactose upstream activation sequences, hence drives the downstream target gene, A β 42 to be expressed throughout <i>Drosophila</i> whole body in a spatiotemporal manner.	14
3.1 Setup for the negative-geotaxis climbing assay, where female <i>Drosophila melanogaster</i> were housed in three empty vials according to different treatment conditions: from the right is Oregon-R: wild-type strain <i>Drosophila</i> as positive control (n = 10); in the middle is Act5C-A β 42: untreated <i>Drosophila</i> AD model as negative control (n = 10); from the left is Act5C-A β 42.DR9: <i>Drosophila</i> AD model treated with <i>Lactobacillus fermentum</i> DR9 (n = 10). (A) The vials were quickly tapped for a few times to knock flies to the bottom, in order to induce the negative geotaxis response of flies by performing climbing action from (B) the bottom to (C) the top of the vial within 10 seconds.	23
4.1 Survival assay, where the effect of the administration of probiotic <i>Lactobacillus fermentum</i> DR9 on survival percentage of female <i>Drosophila melanogaster</i> AD model was evaluated. Oregon-R (○): wild-type strain <i>Drosophila</i> as positive control (n = 50); Act5C-A β 42 (●): untreated <i>Drosophila</i> AD model as negative control (n = 50); Act5C-A β 42.DR9 (◐): <i>Drosophila</i> AD model treated with <i>Lactobacillus fermentum</i> DR9 (n = 50). Results were expressed as mean; error bars (SD); N = 150. ^{A-B} Means of survival percentage of female <i>Drosophila melanogaster</i> for three groups, with different superscripted uppercase letters, are significantly different ($p < 0.05$). Statistical analysis was performed with two-way ANOVA.	27

List of Figures (Continued).

Figure Caption	Page
4.2 Negative-geotaxis climbing assay, where the effect of a 20-days administration (Day 5 (□), Day 10 (▒), Day 15 (■), Day 20 (■)) of probiotic <i>Lactobacillus fermentum</i> DR9 on percentage of flies able to cross half-height of the vial in 5 seconds for female <i>Drosophila melanogaster</i> AD model was evaluated. Oregon-R: wild-type strain <i>Drosophila</i> as positive control (n = 10); Act5C-Aβ42: untreated <i>Drosophila</i> AD model as negative control (n = 10); Act5C-Aβ42.DR9: <i>Drosophila</i> AD model treated with <i>Lactobacillus fermentum</i> DR9 (n = 10). Results were expressed as mean; error bars (SD); N = 30. All mean comparisons were made by days in each group. ^{A-C} Means of percentage of flies crossed half-height of the vial in 5 seconds for female <i>Drosophila melanogaster</i> compared among days in each group, with different <i>superscripted uppercase letters</i> , are significantly different ($p < 0.05$). Statistical analysis was performed with one-way ANOVA.	31
4.3 Negative-geotaxis climbing assay, where the effect of a 20-days administration (Day 5 (□), Day 10 (▒), Day 15 (■), Day 20 (■)) of probiotic <i>Lactobacillus fermentum</i> DR9 on speed for climbing to the top for female <i>Drosophila melanogaster</i> AD model was evaluated. Oregon-R: wild-type strain <i>Drosophila</i> as positive control (n = 10); Act5C-Aβ42: untreated <i>Drosophila</i> AD model as negative control (n = 10); Act5C-Aβ42.DR9: <i>Drosophila</i> AD model treated with <i>Lactobacillus fermentum</i> DR9 (n = 10). Results were expressed as mean; error bars (SD); N = 30. All mean comparisons were made by days in each group. ^{A-B} Means of speed for climbing to the top for female <i>Drosophila melanogaster</i> compared among days in each group, with different <i>superscripted uppercase letters</i> , are significantly different ($p < 0.05$). Statistical analysis was performed with one-way ANOVA.	37
4.4 Negative-geotaxis climbing assay, where the effect of a 20-days administration (Day 5 (□), Day 10 (▒), Day 15 (■), Day 20 (■)) of probiotic <i>Lactobacillus fermentum</i> DR9 on frequency of falling (based on the number of flies show signs of falling) for female <i>Drosophila melanogaster</i> AD model was evaluated. Oregon-R: wild-type strain <i>Drosophila</i> as positive control (n = 10); Act5C-Aβ42: untreated <i>Drosophila</i> AD model as negative control (n = 10); Act5C-Aβ42.DR9: <i>Drosophila</i> AD model treated with <i>Lactobacillus fermentum</i> DR9 (n = 10). Results were expressed as mean; error bars (SD); N = 30. All mean comparisons were made by days in each group. ^A Means of frequency of falling for female <i>Drosophila melanogaster</i> compared among days in each group, with same <i>superscripted uppercase letters</i> , are insignificantly different ($p > 0.05$). Statistical analysis was performed with one-way ANOVA.	40

List of Figures (Continued).

Figure Caption	Page
4.5 Negative-geotaxis climbing assay, where the effect of a 20-days administration (Day 5 (□), Day 10 (▒), Day 15 (■), Day 20 (■)) of probiotic <i>Lactobacillus fermentum</i> DR9 on total distance travelled to the top (based on how far the flies travel to reach to the top) by female <i>Drosophila melanogaster</i> AD model was evaluated. Oregon-R: wild-type strain <i>Drosophila</i> as positive control (n = 10); Act5C-Aβ42: untreated <i>Drosophila</i> AD model as negative control (n = 10); Act5C-Aβ42.DR9: <i>Drosophila</i> AD model treated with <i>Lactobacillus fermentum</i> DR9 (n = 10). Results were expressed as mean; error bars (SD); N = 30. All mean comparisons were made by days in each group. ^{A-B} Means of total distance travelled to the top for female <i>Drosophila melanogaster</i> compared among days in each group, with different <i>superscripted uppercase letters</i> , are significantly different ($p < 0.05$). Statistical analysis was performed with one-way ANOVA.	43
4.6 Negative-geotaxis climbing assay, where the effect of a 20-days administration (Day 5 (□), Day 10 (▒), Day 15 (■), Day 20 (■)) of probiotic <i>Lactobacillus fermentum</i> DR9 on negative geotactic index (the measure of a fly's negative geotactic response to gravity during locomotion) for female <i>Drosophila melanogaster</i> AD model was evaluated. For negative geotactic index, the score is 1 if all flies show negative geotactic response by moving against gravity to the top of the vial, while the score is 0 if all flies loss negative geotactic response by moving along gravity from the top to the bottom of the vial after have reached the top. Oregon-R: wild-type strain <i>Drosophila</i> as positive control (n = 10); Act5C-Aβ42: untreated <i>Drosophila</i> AD model as negative control (n = 10); Act5C-Aβ42.DR9: <i>Drosophila</i> AD model treated with <i>Lactobacillus fermentum</i> DR9 (n = 10). Results were expressed as mean; error bars (SD); N = 30. All mean comparisons were made by days in each group. ^{A-B} Means of negative geotactic index for female <i>Drosophila melanogaster</i> compared among days in each group, with different <i>superscripted uppercase letters</i> , are significantly different ($p < 0.05$). Statistical analysis was performed with one-way ANOVA.	46

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Caption
α	Alpha
β	Beta
$^{\circ}\text{C}$	Degree Celcius
γ	Gamma
<	Less than
>	More than
%	Percent
\pm	Plus-minus
n	Sample size for each group
*	Statistical significance
N	Total sample size

Abbreviation	Caption
Act5C	Actin 5C
A β	Amyloid beta
AD	Alzheimer's disease
AMPK	Adenosine monophosphate-activated kinase
ANOVA	Analysis of variance
APP	Amyloid precursor protein
CFU	Colony-forming unit
dAPPI	APP ortholog of <i>Drosophila</i>
<i>df</i>	Degree of freedom

DR9	<i>Lactobacillus fermentum</i> DR9
elav	Embryonic lethal abnormal vision
Eq.	Equation
FAO	Food and Agriculture Organization
Fig	Figure
<i>g</i>	Gravity
GAL4	Yeast transcription factor for galactose metabolism
GMR	Glass multiple reporter
HSD	Honest Significant Difference
IHGSC	International Human Genome Sequencing Consortium
LAB	Lactic acid bacteria
ml	Millilitre
mm	Millimetre
N/A	Not applicable
<i>p</i>	Probability
pH	Power of hydrogen
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
s	Second
SD	Standard deviation
UAS	Upstream activation sequence
v/v	Volume per volume
w/v	Weight per volume
WHO	World Health Organization

KESAN PROBIOTIK LACTOBACILLI PADA KETAHANAN HIDUP DAN MOBILITI *DROSOPHILA MELANOGASTER* BETINA DENGAN PENYAKIT ALZHEIMER

ABSTRAK

Penyakit Alzheimer (AD), jenis penyakit neurodegeneratif berkaitan penuaan yang paling umum, berkembang dalam bentuk yang tidak berbalik sambil menghampiri perkadaran epidemik tanpa mempunyai ubat atau rawatan pencegahan yang tersedia. Strain probiotik *Lactobacillus fermentum* DR9 dilaporkan menunjukkan kesan antipenuaan pada jangka hayat dan otot pada tikus dengan penuaan teraruh, serta potensi pembalikan AD dengan meringankan neurodegenerasi dalam mata *Drosophila* dengan AD teraruh. Dengan menggunakan model *Drosophila* AD teraruh dengan ekspresi A β 42 manusia melalui sistem UAS/GAL4, kajian ini dijalankan untuk menilai kesan pemberian probiotik pada ketahanan hidup dan mobiliti *Drosophila melanogaster* betina dengan AD. Penilaian dari segi asai ketahanan hidup menunjukkan bahawa, pemberian *L. fermentum* DR9 selama 24 hari telah menambah baik ketahanan hidup dengan meningkatkan peratusan kemandirian model *D. melanogaster* betina AD, berbanding dengan kawalan yang tidak dirawat. Penilaian asai pemanjatan geotaksis negatif menunjukkan bahawa, pemberian *L. fermentum* DR9 selama 20 hari telah menambah baik mobiliti model *D. melanogaster* betina AD dari segi kebolehan memanjat dan kelajuan memanjat, iaitu mobilitinya menurun pada kadar yang lebih perlahan dan tetap berbanding dengan kawalan yang tidak dirawat. Tambahan pula, kekerapan kejatuhan lalat seperti yang ditunjukkan pada model *D. melanogaster* betina AD telah dikurangkan hingga separuh, dengan peningkatan yang ketara dalam jumlah jarak pergerakan ke atas, sementara mengekalkan indeks geotaktik negatif tinggi yang malar dengan rawatan probiotik. Kajian ini membekalkan

pencelikan kepada potensi *L. fermentum* DR9 dalam mengerjakan kesan antipenuaan untuk meringankan simptom AD berkaitan penuaan dari aspek ketahanan hidup dan mobiliti.

EFFECTS OF A LACTOBACILLI PROBIOTIC ON SURVIVAL AND MOBILITY OF FEMALE *DROSOPHILA MELANOGASTER* WITH ALZHEIMER'S DISEASE

ABSTRACT

Alzheimer's disease (AD), as the most prevalent form of aging-associated neurodegenerative disease, progressed in an irreversible manner while approaching epidemic proportions with no cure or preventative therapy yet available. The probiotic strain *Lactobacillus fermentum* DR9 has been reported for exhibiting anti-aging effects on lifespan and muscle in senescence-induced rats, with AD-reversal potential by alleviating neurodegeneration in the eye of AD-induced *Drosophila*. Using an inducible *Drosophila* AD model by human A β 42 expression with UAS/GAL4 system, the study was carried out to evaluate the effects of probiotic administration on the survival and mobility of female *Drosophila melanogaster* with AD. Evaluated by survival assay, the administration of *L. fermentum* DR9 for 24 days improved survival by increasing the survival percentage of female *D. melanogaster* AD model, as compared to the untreated control. Evaluated by the negative-geotaxis climbing assay, the administration of *L. fermentum* DR9 for 20 days improved the mobility of female *D. melanogaster* AD model in terms of climbing ability and climbing speed, by declining at a slower and more constant rate as compared to the untreated control. Additionally, the frequency of flies falling as shown in female *D. melanogaster* AD model has decreased to a half, with significant increase in the total distance travelled to the top, while retained a constant high negative geotactic index with probiotic treatment. This study provided an insight into the potential of *L. fermentum* DR9 in exerting anti-aging effects to alleviate the aging-associated AD symptoms from the aspect of survival and mobility.