

**EFFECT OF A LACTOBACILLI PROBIOTIC ON
LONGEVITY AND MOBILITY OF MALE
DROSOPHILA MELANOGASTER WITH
ALZHEIMER'S DISEASE**

LIM CHIN PENG

**UNIVERSITI SAINS MALAYSIA
JUNE 2020**



USM UNIVERSITI
SAINS
MALAYSIA



**PUSAT PENGAJIAN TEKNOLOGI INDUSTRI
UNIVERSITI SAINS MALAYSIA**

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

Nama Penyelia: Prof. Liong Min Tze

Bahagian: Teknologi Bioproses

Saya telah menyemak semua pembetulan/pindaan yang dilaksanakan oleh
Encik/Puan/Cik Lim Chin Peng

mengemui 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

15/7/2020

Tarikh



**EFFECT OF A LACTOBACILLI PROBIOTIC ON
LONGEVITY AND MOBILITY OF MALE
DROSOPHILA MELANOGASTER WITH
ALZHEIMER'S DISEASE**

by

LIM CHIN PENG

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 institution.



.....

LIM CHIN PENG

JUNE 2020

ACKNOWLEDGEMENTS

I would like to forward my gratitude to my supervisor, Prof. Liong Min Tze for providing the opportunity to engage in a new research field including breeding fruit flies and performing experiments that I have never involved in my final year project. She was always concern about the progress and problems that were faced throughout the project followed by the valuable feedbacks given. She would inspire me to think creatively so that I can solve issues faced or achieve goals critically and independently as well.

Next, I am grateful to my co-supervisor, Dr. Mohd. Ghows bin Mohd. Azzam from the School of Biological Sciences. Thank you for allowing us to go to your lab to breed flies and conduct experiments. I am thankful for the useful guidance that you have provided whenever we needed.

Also, I would want to say thank you to research officers Mr Ahmad Imran Zaydi and Mr Lee Boon Kiat, and postgraduate students Ms. Florence Tan Hui Ping and Mr. Andrew Chung Jie Ting who willingly and selflessly shared your experiences in conducting experiments and solving problems as well as on the operation of Toxtrac software. I also appreciated all the guidance and encouragement from all of you.

Lim Chin Peng

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	xi
Abstrak	xiii
Abstract	xv
CHAPTER 1 INTRODUCTION	
1.1 Research Background	1
1.2 Problem Statement	4
1.3 Research Scope and Objectives	5
CHAPTER 2 LITERATURE REVIEW	
2.1 The Relation Between Aging and Alzheimer's Disease	7
2.2 <i>Drosophila melanogaster</i> as A Model of Alzheimer's Disease	9
2.3 Activation of Gene Expression in <i>Drosophila melanogaster</i> Using UAS-GAL4 System	11
2.4 Amyloid Aggregation Leading to the Development of Alzheimer's Disease	13
2.5 Longevity and Mobility of <i>Drosophila</i> model of Alzheimer's Disease	15
2.6 Health Benefits of Probiotics Against Alzheimer's Disease	17
CHAPTER 3 MATERIALS AND METHODS	
3.1 Breeding of <i>Drosophila</i> Stocks	21
3.2 Preparation of Feed	22
3.3 Preparation of Bacterial Strain and Cultures	22
3.4 Survival Assay	23
3.5 Climbing Assay	23
3.6 Statistical Analysis	25
CHAPTER 4 RESULTS AND DISCUSSION	
4.1 Assessment of Longevity of Male <i>Drosophila melanogaster</i>	26
4.2 Assessment of Mobility of Male <i>Drosophila melanogaster</i>	29

4.2.1	Average Speed Climbed to Reach The Top Of Vial	30
4.2.2	Total Distance Climbed	32
4.2.3	Time Taken for Half Population to Cross The Centre Line	34
4.2.4	Average Height Climbed	36
4.2.5	Negative Geotaxis Behaviour	38
4.2.6	Frequency of Appearance in Zones	40
4.2.6a	Top Zone	40
4.2.6b	Middle Zone	42
4.2.6c	Bottom Zone	43
4.2.7	Immobility	45
4.2.8	Percentage of Falling	47

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

FOR FUTURE RESEARCH

5.1	Conclusion	49
5.2	Recommendations for Future Research	51

REFERENCES

APPENDICES

LIST OF TABLES

Table	Caption	Page
3.1	List of <i>Drosophila</i> lines and strain of LAB used.	22

LIST OF FIGURES

Figure	Caption	Page
2.1	A simplified illustration of UAS-Gal4 system in which GAL4 expressing male fly crossed with a UAS transgenic female fly fused with a human disease gene. Progenies of these two lines carry both sets of gene sequences. Expression of the human gene relies on the GAL4 gene sequence.	13
3.1	The dimensions of the plastic vial where the height is equally divided into three zones for the determination of frequency of appearance of flies in each zone during climbing test.	24
4.1	Effect of administration of probiotic <i>Lactobacillus fermentum</i> DR9 on the longevity of male <i>Drosophila melanogaster</i> . Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). Results were expressed as mean; error bars (SD); n=450 (150 flies in each of the three groups).	27
4.2	Effect of administration of probiotic <i>Lactobacillus fermentum</i> DR9 on the longevity of male <i>Drosophila melanogaster</i> . Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). D: P= <0.001, G: P= 0.151, DxG: P= 1.000. D: effect of days; G: effect of test group of male <i>Drosophila</i> ; DxG: interaction between day and test group. ^{A,B} Means of the longevity of three groups of male <i>Drosophila melanogaster</i> , with different <i>superscripted uppercase letters</i> are significantly different (P < 0.05). Results were expressed as mean; error bars (SD); n=150 (50 flies in each of the three groups). Statistical analysis was performed using two-way ANOVA.	29
4.3	Effect of 20-day administration of probiotic <i>Lactobacillus fermentum</i> DR9 on the average speed of male <i>Drosophila melanogaster</i> climbed to the top of vial. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). ^{A-C} Means of the average speed of three groups of male <i>Drosophila melanogaster</i> climbed to the top of vial, with different <i>superscripted uppercase letters</i> are significantly different (P < 0.05). Results were taken at fifth day (■), tenth day (■), fifteenth day (■) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA.	31

- 4.4 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the total distance climbed by male *Drosophila melanogaster*. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). ^{A-C}Means of the total distance climbed by three groups of male *Drosophila melanogaster*, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA. 33
- 4.5 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the time taken for half population of male *Drosophila melanogaster* to cross the center line of the vial during climbing test. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). ^{A,B}Means of the frequency of three groups of male *Drosophila melanogaster* appeared at bottom zone, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA. 35
- 4.6 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the average height climbed by male *Drosophila melanogaster* in 2 seconds after the tapping of vial. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). ^{A-C}Means of the frequency of three groups of male *Drosophila melanogaster* appeared at bottom zone, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA. 38
- 4.7 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the percentage of male *Drosophila melanogaster* having negative geotaxis track during climbing test. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). ^{A-C}Means of the frequency of three groups of male *Drosophila melanogaster* appeared at bottom zone, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at 39

- fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA.
- 4.8 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the frequency of male *Drosophila melanogaster* appeared at top zone during climbing test. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). ^{A,B}Means of the frequency of three groups of male *Drosophila melanogaster* appeared at top zone, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA. 41
- 4.9 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the frequency of male *Drosophila melanogaster* appeared at middle zone during climbing test. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). ^{A,B}Means of the frequency of three groups of male *Drosophila melanogaster* appeared at middle zone, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA. 43
- 4.10 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the frequency of male *Drosophila melanogaster* appeared at bottom zone during climbing test. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-Aβ42.nf (■) served as negative control. Actin5C-Aβ42.DR9 was treated with probiotic (▲). ^{A,B}Means of the frequency of three groups of male *Drosophila melanogaster* appeared at bottom zone, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA. 44
- 4.11 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the percentage of immobile male *Drosophila melanogaster* during climbing test. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-

A β 42.nf (■) served as negative control. Actin5C-A β 42.DR9 was treated with probiotic (▲). ^{A,B}Means of the percentage of three groups of immobile male *Drosophila melanogaster*, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA.

- 4.12 Effect of 20-day administration of probiotic *Lactobacillus fermentum* DR9 on the percentage of male *Drosophila melanogaster* fell during climbing test. Wild type flies (●) are Oregon-R strain and served as positive control while Actin5C-A β 42.nf (■) served as negative control. Actin5C-A β 42.DR9 was treated with probiotic (▲). ^{A,B}Means of the percentage of three groups of male *Drosophila melanogaster* fell, with different *superscripted uppercase letters* are significantly different ($P < 0.05$). Results were taken at fifth day (■), tenth day (▒), fifteenth day (▓) and twentieth day (□) and expressed as mean; error bars (SD); n=90 (30 flies in each of the three groups). Statistical analysis was performed using one-way ANOVA. 48

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Caption
α	Alpha
β	Beta
\$	Dollar sign
γ	Gamma
κ	Kappa
μ	Micro
%	Percent

Abbreviations	Caption
A β 42	Amyloid beta 42 peptide
AD	Alzheimer's disease
ADI	Alzheimer's Disease International
AMPK	Adenosine monophosphate-activated kinase
ANOVA	Analysis of variance
APP	Amyloid precursor protein
BACE	Beta-secretase
BBB	Blood-brain-barrier
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
cfu	Colony-forming unit
cm	Centimetre
CNS	Central nervous system
CREB	cAMP response element-binding protein
dAPPI	Drosophila APP-like
DNA	Deoxyribonucleic acid
DR9	<i>Lactobacillus fermentum</i> DR9
GABA	γ -aminobutyric acid
GRAS	Generally regarded as safe
HSD	Honestly significant difference
IGF	Insulin-like growth hormone
kg	Kilogram

KGGR	Kyoto Drosophila Genome and Genetic Resource
LAB	Lactic acid bacteria
LTP	Long-term potentiation
MDA	Malondialdehyde
mGluRs	Metabotropic glutamate receptors
mm	Millimetre
MMSE	Mini-mental state examination
MRS	De Mann, Rogosa, and Sharp
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDAR	Anti-N-Methyl-D-Aspartate Receptor
PS	Presenilin
PUFA	Polyunsaturated fatty acids
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SPSS	Statistical Package for the Social Science
UAS	Upstream activation sequence
WHO	World Health Organization

KESAN PROBIOTIK LACTOBACILLI TERHADAP TEMPOH HAYAT DAN MOBILITI *DROSOPHILA MELANOGASTER* JANTAN YANG MENGHIDAPI PENYAKIT ALZHEIMER

ABSTRAK

Kira-kira 50 juta orang di dunia didiagnosis dengan penyakit Alzheimer seperti yang dilaporkan pada tahun 2019. Kerosakan lokomotor berlaku pada peringkat awal penyakit dan penurunan prestasi pesakit selalunya berkaitan rapat dengan penuaan. Tiada rawatan yang berkesan lagi dapat dijalankan secara meluas walaupun banyak kajian saintifik telah dilakukan. Kajian ini bertujuan untuk menilai kesan *Lactobacillus fermentum* DR9 terhadap tempoh hayat *Drosophila melanogaster* yang menghidapi penyakit Alzheimer dan mengkaji kesan *Lactobacillus fermentum* DR9 terhadap mobiliti *Drosophila melanogaster* yang menghidapi penyakit Alzheimer. Kajian dari segi tempoh hayat dan kemampuan pendakian telah dijalankan dalam peniga untuk mengetahui kesan rawatan menggunakan *Lactobacillus fermentum* DR9 dalam mengurangkan kadar pemendekan jangka hayat dan penurunan mobiliti *Drosophila melanogaster* yang menghidapi penyakit Alzheimer. Berdasarkan hasil kajian, tempoh hayat Actin5C-A β 42.DR9 menunjukkan penurunan yang lebih perlahan dan menyimpang daripada Actin5C-A β 42.nf mulai hari ke-11. Pada hari ke-23, tempoh hayat Actin5C-A β 42.DR9 adalah 23% lebih tinggi daripada Actin5C-A β 42.nf. Kebanyakan aspek pendakian menunjukkan hasil kajian yang menarik bahawa Actin5C-A β 42.DR9 mengalami peningkatan yang baik dari hari kelima sehingga hari ke-20 selepas menerima rawatan probiotik. Perkaitan antara aspek-aspek pendakian turut menyokong bahawa rawatan probiotik dapat dipertimbangkan untuk mengurangkan kemerosotan mobiliti. Penurunan kelajuan pendakian Actin5C-A β 42.DR9 telah dikurangkan secara ketara, seterusnya menyumbang kepada waktu

yang lebih pendek untuk melintasi garis tengah dan juga ketinggian yang lebih tinggi berbanding dengan Actin5C-A β 42.nf. Kami yakin bahawa rawatan probiotik menggunakan *L. fermentum* DR9 berpotensi untuk menyelamatkan keadaan pemendekan tempoh hayat dan kemerosotan mobiliti *Drosophila melanogaster* yang menghadapi penyakit Alzheimer.

EFFECT OF A LACTOBACILLI PROBIOTIC ON LONGEVITY AND MOBILITY OF MALE DROSOPHILA MELANOGASTER WITH ALZHEIMER'S DISEASE

ABSTRACT

Approximately 50 million people are diagnosed with Alzheimer's disease (AD) in the world as reported in 2019. Locomotor impairment were present in the early stage of the disease and the decline in the performance of AD patients is often related to aging. Effective treatment has yet to be conducted extensively even though considerable amounts of studies had been performed. This study aimed to evaluate the effect of *Lactobacillus fermentum* DR9 on the longevity of AD flies and to study the effect of *Lactobacillus fermentum* DR9 on the mobility of AD flies. Longevity test and climbing test were conducted in triplicates to determine the effect of treatment using *Lactobacillus fermentum* DR9 in alleviating the lifespan shortening and mobility impairment of AD flies respectively. Based on the results, the survival rate of Actin5C-A β 42.DR9 had showed a slower decreasing trend that deviated from that of Actin5C-A β 42.nf from Day 11 onwards. At Day 23, the survival rate of Actin5C-A β 42.DR9 was 23% higher than that of Actin5C-A β 42.nf. Most climbing parameters presented appealing results inferring that Actin5C-A β 42.DR9 had good improvement after receiving probiotic treatment from Day 5 to Day 20. The correlations among climbing parameters even supported that probiotic treatment can be put into consideration to alleviate the mobility degeneration. It was shown that the decrease in climbing speed of Actin5C-A β 42.DR9 was significantly attenuated, consequently resulting in shorter time required to cross the centre line and more height climbed than Actin5C-A β 42.nf. We believed that probiotic treatment using *L. fermentum* DR9 has the potential to rescue the shortening of lifespan and mobility impairment of AD model.