



UNIVERSITI PUTRA MALAYSIA

**ISOLATION OF GLUTAMIC ACID-PRODUCING LACTIC ACID
BACTERIA AND ITS APPLICATION IN *THOSAI***

MOHSEN ZAREIAN

FSTM 2011 28

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By

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MASTER OF SCIENCE

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2011

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AND ITS APPLICATION IN *THOSAI***

By

MOHSEN ZAREIAN

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

November 2011

An abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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November 2011

Chairperson: Professor Nazamid Saari, PhD

Faculty: Food Science and Technology

In the present study, six different fermented foods were evaluated as potential source for the isolation and characterization of glutamic acid-producing lactic acid bacteria. A total of two hundred and seventy isolates were screened sequentially for catalase activity and Gram-staining, out of which, 218 were categorized as lactic acid bacteria (LAB). Microscopic and biochemical tests were used to further identify and authenticate these 218 presumptive LAB strains. The results of the HPLC analysis revealed that only 35 strains, out of 218, have glutamic acid producing ability. The highest glutamic acid production potential was exhibited by the strain TMP 3b85, isolated from *tempeh* (fermented soybean). Further tests involving the use of 16S rRNA gene sequencing and sugar assimilation assay identified TMP 3b85 as *Lactobacillus plantarum*. Time-course

analysis of the culture medium revealed the glutamic acid production ability of TMP 3b85 to be maximum after 96 h. In addition, characteristics of *L.plantarum* such as growth rate, glucose consumption and pH profile affecting the yield of glutamic acid during fermentation were also evaluated. The fermentation process parameters such as pH, temperature, carbon source (glucose) and nitrogen source (ammonium nitrate) were optimized through factorial design and Response Surface Methodology to obtain the highest yield of glutamic acid in a basal medium. The highest glutamic acid level (3.353 mM) was obtained under the following optimized conditions: pH, 4.5; temperature, 37 °C; glucose, 12%; ammonium nitrate, 0.7%. In order to investigate glutamic acid production by *L.plantarum* in a food system, *thosai* was chosen as a substrate. *L.plantarum* (4.36×10^7 CFU/ml) was inoculated into the fermentor containing *thosai* ingredients including 29.7 g rice; 45 g wheat flour and 9.9 g skim milk powder in 84.6 ml distilled water. Fermentation was performed at ambient room temperature (29 °C); agitation rate 150 rpm for 216 h. Highest yield of glutamic acid was obtained (277 mg/kg) after 120 h. The findings of this study provide a potential basis for exploiting selected fermented food-related LAB as an alternative source for production of glutamic acid as a precursor of γ -amino butyric acid.

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

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Oleh

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November 2011

Pengerusi : Profesor Nazamid Saari, PhD

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Dalam kajian ini, bacteria asid laktat berkebolehan menghasilkan asid glutamat telah diasing dan di cirikan daripada enam sumber makanan fermentasi yang berbeza dan berpotensi. Dua ratus tujuh puluh isolat telah disaring secara berperingkat bagi aktiviti katalase dan kaedah pewarnaan gram, dimana daripada jumlah keseluruhan, 218 telah dikategorikan sebagai bacteria asid laktik. Ujian mikroskopik dan biokimia telah digunakan bagi mengenal pasti dan mengesahkan 218 strain LAB. Keputusan analisis oleh HPLC menunjukkan bahawa, hanya 35 strain daripada 218, mempunyai kebolehan dalam menghasilkan asid glutamik. Bacteria pengeluar asid glutamik tertinggi dan mempunyai potensi telah ditunjukkan oleh strain TMP 3b85, diperolehi daripada *tempeh* (kacang soya yang ditapai). Ujian lanjutan yang melibatkan penggunaan jujukan gen 16s RNA dan ujian asimilasi gula telah mengenal pasti TMP 3b85 sebagai *Lactobacillus*

plantarum. Analisis selang waktu medium kultur menunjukkan bahawa kebolehan dalam menghasilkan asid glutamik oleh TMP 3b85 akan mencapai kepekatan maksimum selepas 96 jam. Selain itu, ciri-ciri *Lactobacillus plantarum* seperti kadar pertumbuhan, penggunaan glukosa dan profil pH yang memberi kesan terhadap hasil asid glutamik semasa proses penapaian juga dinilai. Parameter proses penapaian seperti pH, suhu, sumber karbon (glukosa) dan sumber nitrogen (ammonium nitrat) dioptimumkan melalui reka bentuk faktorial dan Rancangan Metodologi Permukaan untuk mendapat hasil tertinggi asid glutamik dalam medium basal. Kadar tertinggi asid glutamik (3.353 mM) telah dibawah mengikut keadaan optimum seperti berikut: pH, 4.5, suhu 37 °C, glukosa 12%; dan ammonium nitrat, 0.7%. Dalam usaha untuk menyiasat pengeluaran asid glutamik oleh *L. plantarum* dalam sistem makanan, tosa telah dipilih sebagai substrat. *L. plantarum* (4.36×10^7 CFU/ml) telah inokula gikanke dalam bioreaktor yang mengandungi bahan-bahan tosa seperti 29.7 g nasi; 45 g tepung gandum dan 9.9 g serbuk susu tanpa lemak dalam 84.6 ml air suling. Penapaian telah dilakukan pada suhu ambien (29°C); kadar pengacauan 150 rpm selama 216 jam. Hasil Tertinggi asid glutamik telah diperolehi (277 mg/kg) selepas 120 jam. Penemuan kajian ini memberikan potensi asas bagi mengeksploitasi makanan terpilih yang mengandungi LAB untuk dijadikan sumber alternatif bagi menghasilkan asid glutamat sebagai pelopor kepada pengeluaran GABA.

ACKNOWLEDGEMENT

I would like to thank all the people who made this project possible. Thanks to my supervisor, Professor Dr. Nazamid Saari for his continuous support, guidance, supervision, valuable advice and constructive comments. He has sacrificed much of his time and expertise contributing towards improvement of studies. Sincere thanks to the members of my committee specially Dr. Abdul Karim Sabo Mohamed, for all his advice and contributions throughout this project. Special thanks to Dr. Afshin for the helpful discussion.

A special appreciation goes to Mr. Halim from the HPLC laboratory and also Mr. Zulkefli from the Microbiology Laboratory. Thanks also to Mr. Azman and all the laboratory assistants in the Biochemistry Laboratory due to sharing all kinds of experience with me. Not forgetting Ms. Suraya and also Ms. Liza. All of them contributed selflessly and willingly throughout the duration of my project. I do appreciate all of their advice and assistance given to me.

Thanks to all my friends in the Food Biotechnology and Functional Food Research Laboratory, Farrah, Farnaz, Babak, Amir and Negar.

To my parents, sister and brother, thank you all for the encouragement.

Approval

I certify that an Examination Committee met on **date of viva** to conduct the final examination of **Mohsen Zareian** on his Master of Science thesis entitled “**Isolation of Glutamic Acid-Producing Lactic Acid Bacteria and Its Application in Thosai**” in accordance with Universiti Pertanian Malaysia (Higher degree) Act 1980 and Universiti Pertanian Malaysia (Higher degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

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

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Date: 29 November 2011

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENT	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATION	xvi
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	3
2.1 Amino Acids	3
2.2 Glutamic Acid	3
2.2.1 Glutamic Acid-Producing Microorganisms	4
2.2.2 Mechanism of Glutamic Acid Production in Microorganisms	5
2.2.3 Optimization of Glutamic Acid	9
2.2.4 Response Surface Methodology	11
2.2.5 Methods for Measuring Glutamic Acid	14
2.3 Lactic Acid Bacteria	16
2.3.1 Importance of Lactobacilli in Foods	18
2.3.2 The Genus <i>Lactobacillus</i>	19
2.3.3 Screening and Identification of LAB	21
2.4 Polymerase Chain Reaction	23
2.5 16S ribosomal RNA Gene Sequencing	29
2.6 Fermentation Technology	30
2.6.1 Food Fermentation	32
2.6.2 Traditional Food Fermentation	34
2.6.3 Biological Value of Fermented Foods	35
2.6.4 Examples of Fermented Foods	35
3 MATERIAL AND METHODS	41
3.1 Materials	41
3.1.1 Fermented Food Samples	41
3.1.2 Chemicals and Media	41
3.2 Methods	41
3.2.1 Extraction of Glutamic Acid from Food Samples	41
3.2.2 Extraction of Glutamic Acid in MRS Broth	42
3.2.3 Quantitative Analysis of Glutamic Acid	43
3.2.4 Isolation of LAB	44
3.2.5 Screening Glutamic Acid-Producing LAB	45
3.2.6 Phenotypic Identification of LAB	45
3.2.7 Genotypic Identification of LAB	48

3.2.8	Time-Course Study of Glutamic Acid Production	50
3.2.9	Study of the pH Profile	50
3.2.10	Growth Profile Study	50
3.2.11	Effect of Various Carbon Sources on the Glutamic Acid Production	51
3.2.12	Study of Reducing Sugar Profile	51
3.2.13	RSM Design	52
3.2.14	Preparation of <i>Thosai</i> as a Food System	56
3.2.15	Study of Glutamic Acid Production in <i>Thosai</i>	57
3.2.16	Statistical analysis	57
4	RESULTS AND DISCUSSION	59
4.1	Glutamic Acid Content in Food Samples	59
4.2	Isolation of LAB from Food Samples	63
4.3	Screening Glutamic Acid-Producing LAB	65
4.4	Identification of the LAB Strain TMP 3b85	68
4.4.1	Phenotypic Identification	68
4.4.2	Genotypic Identification	72
4.4.3	Study of Glutamic Acid-Production Profile	76
4.4.4	Study of pH Profile	78
4.4.5	Growth Characteristics of the LAB Strain TMP 3b85	80
4.5	Optimization Study	82
4.5.1	Effect of Various Carbon Sources on Glutamic Acid Production	82
4.5.2	Study of Glucose Consumption	85
4.5.3	Response Surface Methodology	86
4.6	Glutamic Acid Formation in a Food System	100
4.7	Study of the pH Change during <i>Thosai</i> Fermentation	101
5	CONCLUSION AND RECOMMENDATION FOR FUTUR STUDY	104
	REFERENCES	106
	APPENDIX	132
	BIODATA OF STUDENT	143