UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF L-LACTIC ACID USING VARIOUS CARBON SOURCES BY ENTEROCOCCUS GALLINARUM EBI

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FSMB 2002 12

PRODUCTION OF L-LACTIC ACID USING VARIOUS CARBON SOURCES BY *ENTEROCOCCUS GALLINARUM* EB1

By

CHEONG WENG CHUNG

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

October 2002

Specially dedicated to,

My beloved parents who brought me to this world,

my brothers who gave me the encouragements and laughter,

and friends for their invaluable advices and morale supports.

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

PRODUCTION OF L-LACTIC ACID USING VARIOUS CARBON SOURCES BY *ENTEROCOCCUS GALLINARUM* EB1

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This study reports on the production of L-lactic acid using a locally isolated bacteria. Bacteria were screened and isolated from POME sludge, kitchen refuse, leachate biomass, soil and fermented milk. Five bacteria shown positive result in the preliminary test and only one bacteria which was isolated from POME effluent shown the highest lactic acid production. Using shake flask culture, 18.0 g/L of L-lactic acid was produced from 20.0 g/L glucose . The selectivity of lactic acid produced by the bacteria was 99.8% compared to other organic acids. This indicated that the bacteria can be use for the production of L-lactic acid. Using the BIOLOG system, the bacteria was identified belonging to the family *Enterococcus gallinarum* and named as *Enterococcus gallinarum* EB1. Morphologically, the bacteria is cocci-shaped and in chains or grouped. The optimal growth condition for the bacteria was at pH 6 and temperature 37°C where at this condition, the bacteria able to produced highest lactic acid yield at 1.9 g/g using glucose as substrate. The organic acids composition was dependent on the pH and temperature. In an anaerobic batch fermentation to produce lactic acid using four types of

substrates (glucose, kitchen refuse, sago starch and cooked rice), the highest lactic acid production was 45.0 g/L. From the experiment, the bacteria was able to convert the kitchen refuse into lactic acid at 45.2 g/L and small amount of other organic acids. The comparison was also done with other substrates to show that the bacteria able to utilise kitchen refuse in lactic acid production. In the recovery process of lactic acid, the best method was to use H_2SO_4 prior to evaporation at 90°C with 3mmHg vacuum pressure. H_2SO_4 able to free lactic acid from lactate salts formed in the fermentation because the use of NaOH to control pH in the bioreactor throughout the fermentation process. The evaporation method able to achieved 86.76% lactic acid recovery yield from the fermentation broth. It was the highest recovery yield recorded in evaporation compared to other evaporation method with additional of solvents (propanol and butanol) with temperature at 90°C and pressure around 3mmHg.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN ASID L-LAKTIK MENGGUNAKAN PELBAGAI JENIS SUMBER KARBON OLEH *ENTEROCOCCUS GALLINARUM* EB1

Oleh

CHEONG WENG CHUNG

Oktober 2002

Kajian ini melaporkan penggunaan bakteria tempatan dalam pneghasilan asid L-laktik. Penyaringan bakteria dibuat daripada pelbagai bahan dan tempat (sisa POME, sampah sarap, enapcemar luluhan sampah dan susu basi). Lima bakteria telah menunjukkan keputusan positif pada ujian awal dan hanya satu bakteria dipilih iaitu bakteria yang disaring dari sisa POME kerana menunjukkan prestasi yang menggalakkan dalam penghasilan asid L-laktik. Kepekatan 18.0 g/L asid L-laktik dihasilkan di dalam eksperimen kelalang kon menggunakan 20.0 g/L glukosa. Peratusan pemilihan asid laktik pula 99.8% oleh bakteria tersebut berbanding asid organik yang lain. Ini menunjukkan bahawa bakteria ini boleh digunakan dalam penghasilan asid L-laktik. Sistem BIOLOG digunakan untuk mengenalpasti jenis bakteria dan ia adalah daripada famili *Enterococcus gallinarum*, dan dinamakan *Enterococcus gallinarum* EB1. Morfologi bakteria ini adalah berbentuk cocci dan mempunyai ciri-ciri bersambungan antara satu sama lain atau berkelompok. Keadaan pertumbuhan optimum bagi bakteria ini adalah pada pH 6 dengan suhu 37°C dimana keadaan ini merangsangkan bakteria untuk

menunjukkan penghasilan nisbah asid laktik dengan berat kering sel sebanyak 1.919 g/g. Komposisi asid adalah bergantung kepada pH dan suhu. Di dalam fermentasi anaerobik sesekelompok untuk menghasilkan asid laktik dari empat jenis substrat (glukosa, sampah sarap, sago dan nasi), kepekatan asid laktik paling tinggi dicatatkan pada 45.0 g/L. Dari eksperimen ini, bakteria tersebut dapat menukarkan sampah sarap kepada asid organik dan asid laktik pada kepekatan 45.2 g/L dan terdapat sedikit asid organik yang lain dihasilkan. Perbandingan juga dibuat dengan menggunakan substrat berlainan untuk perbandingan prestasi bakteria tersebut untuk penghasilan asid laktik daripada sampah-sarap. Penggunaan H_2SO_4 dalam proses penyulingan pada suhu 90°C dan tekanan 3mmHg adalah cara terbaik. Ini disebabkan oleh asid sulfurik dapat membebaskan asid laktik dari bentuk garam laktat yang terhasil akibat penggunaan NaOH untuk mengawal pH di dalam bioreaktor semasa sepanjang proses fermentasi. Cara penyulingan ini dapat mencatatkan 86.76% hasil perolehan semula asid laktik. Catatan ini merupakan yang tertinggi berbanding dengan cara penyulingan yang mencampurkan propanol dan butanol dengan menggunakan suhu pada 90°C dan tekanan pada 3mmHg.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude and appreciation to my main supervisor, Associate Prof. Dr. Mohd. Ali Hassan and members of the supervisory committee, Prof. Dr. Mohamed Ismail Abdul Karim and Dr. Suraini Abdul Aziz, for their invaluable advice and support, encouragement and willingness to share their views throughout the project. I am personally grateful to Associate Prof. Dr. Mohd Ali Hassan and my supervisory committee for spending time in guiding me with this thesis in order for me to complete the Master degree.

I sincerely thank Associate Prof. Dr. Mohd Ali Hassan for giving me the chance to further my study to postgraduate level two years ago and also for his immeasurable support, advice and ideas throughout my study. I extend my gratitude to Prof. Dr. Mohamed Ismail Abdul Karim for his advice and to Dr. Suraini Abdul Aziz for her help in the project.

In addition, I would like to express my appreciation to my labmates; Phang Lai Yee, Ong Ming Hooi, Nor' Aini Abdul Rahman, Jame'ah Hamed, Norrizan Abdul Wahab, Hafizah Kassim, Manisya Zauri, Abdul Rahman Abdul Razak, Zainal Baharum, Sim Kean Hong, Wong Kok Mun and Zaizuhana Shahrim and fermentation laboratory staffs; Mr. Rosli Aslim, Madam Renuga a/p Panjamurti, Madam Latifah Hussein and

Madam Aluyah Marzuki, thank you for your moral support, cooperation and willingness to teach me during the study.

My heartiest thanks go to my beloved parents and brothers for their patience, support and encouragement. To my friends, Chee Kuan, Shang Der and Kiat Siong, deepest appreciation for their advice, motivation and friendship.

I certify that an Examination Committee met on 22nd. October 2002 to conduct the final examination of Cheong Weng Chung on his Master of Science thesis entitled "**Production Of L-Lactic Acid Using Various Carbon Sources By *Enterococcus gallinarum* EB1**" 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 follows:

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The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement of the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Name : CHEONG WENG CHUNG
Date : 22nd October 2002

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LIST OF ABBREVIATIONS

LAB	-	Lactic acid bacteria
PHA	-	Polyhydroxyalkanoates
PLA	-	Poly-L-lactic acid
NADH	-	Nicotinamide adenine dinucleotide
ATP	-	Phosphoenolpyruvate
PGA	-	Polyglycolic acid
EMP	-	Glycolysis-Embden-Meyerhoff-Parnas pathway
BOD_5	-	Biological Oxygen Demand 5 Days
COD	-	Chemical Oxygen Demand
TKN	-	Total Kjeldahl Nitrogen
POME	-	Palm Oil Mill Effluent
TS	-	Total Solids
PTS	-	Phosphotransferase system
L. lactis	-	*Lactococcus lactis*
BUG	-	BIOLOG Universal Growth
PTS	-	Phospho-Transferase System
E^{II}	-	Enzyme 2

CHAPTER 1

INTRODUCTION

1.0 Lactic Acid Producing Microorganism

Lactic acid bacteria (LABs) belong to a group of Gram-positive anaerobic bacteria that excrete lactic acid as their main fermentation product into the culture medium. LABs were among the first organisms to be used in food manufacturing. Today LABs play crucial roles in the manufacturing of fermented milk products, vegetables and meat, as well as in the processing of other products such as wine. In order to understand and especially to manipulate the roles of these LABs in these fermentation processes, LABs have been studied extensively and are now among the best-characterised microorganisms with respect to their genetics, physiology and applications. The relative simplicity of LABs makes them excellent candidates for complete analysis of the metabolic pathways in the near future. The extensive knowledge gained of LABs has opened new possibilities for their application. Tailor-made LABs with desired physiological traits can be constructed and can be applied to optimize the food manufacturing processes or to manipulate the organoleptic properties.

1.1 Lactic acid and Poly-L-lactic Acid from Kitchen Refuse

Kitchen garbage or refuse is another organic substance that can be subjected to biological treatment for organic acids production, particularly for lactic acid production under controlled conditions. Since kitchen refuse mainly contained cooked waste and remains of meals, it provides rich nutrients including carbohydrate, lipid, protein and other compounds and does not usually contain harmful compounds (Rintala and Birgitte, 1994). These compounds are essential for the growth of microorganisms to synthesize desired products. In developed countries such as Japan, segregation of wastes according to different criteria e.g. combustible and non-combustible material, recyclable materials (bottles, cans, newsprint and paper), has been adopted in the whole country. Kitchen waste is classified as combustible material which is usually subjected to incineration. However, owing to the environment pollution problem, an appropriate method of handling this organic waste has to be developed. Kitchen and restaurant wastes have been utilised as substrates for the production of organic acids. A variety of organic acids at different concentrations could be produced under different fermentation conditions. The organic acids were then converted to bacterial biopolymers or polyhydroxyalkanoates (PHA). Another strategy in the utilisation of such wastes is to produce L-lactic acid which can be used for the production of polylactate or PLA. This is another kind of bioplastic which is gaining popularity due to its superior physical strength and longer

durability compared to PHA.

Recently, lactic acid fermentation has received much attention because of increasing demands for new bioengineering materials such as biodegradable lactide polymers and the high cost of petroleum which is usually used as feed stock for production of lactic acid in the conventional chemical processes.

1.2 Objectives

The scope of this study focused on development of process for establishing high performance L-lactic acid fermentation using local isolate. Therefore, the objectives of this research are;

1. To screen local bacteria strain for the production of pure L-lactic acid from various sources
2. To investigate the effect of the culture pH and temperature on lactic acid production
3. To perform kinetic studies for optimization of L-lactic acid fermentation