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ANTIBACTERIAL ACTIVITIES OF LACTIC ACID BACTERIA ISOLATED FROM ROTTEN VEGETABLES

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "**Antibacterial Activities of Lactic Acid Bacteria Isolated from Rotten Vegetables**" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh **Lee Pei Zhen (162958)** sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Rising trend of foodborne illness and emergence of antimicrobial resistance in Malaysia pose serious threat to the public and thus a new and effective antibacterial agent is required to address the situation. The non-pathogenic lactic acid bacteria (LAB) with high antibacterial potential can be isolated from rotten vegetables and showed inhibitory activity toward foodborne pathogens. The objective of this study was to isolate and identify LAB, also to determine the inhibitory activity from rotten vegetables against foodborne pathogens. A total of eight LAB isolates were isolated from seven types of rotten vegetables. The antibacterial activities of isolates against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* L10 were determined using the agar-well diffusion method. *E. coli* ATCC 8739 appeared to be the most susceptible test organisms to LAB isolates as the inhibition zone of all LAB isolates against *E. coli* ATCC 8739 were obviously larger than the other test organisms. The antibacterial agents were stable within limit range of pH values from pH 2.0 to 5.0. Antibacterial activity was not observed when the pH of the supernatant was adjusted to above pH 6.0. Inhibitory activity of isolates can be observed even at high temperature from 50^{o}C to 100^{o}C, yet the zone of inhibition showed a decrease trend in all isolates as the temperature goes up.

ABSTRAK

Peningkatan trend penyakit bawaan makanan dan kemunculan rintangan antimikrob di Malaysia menimbulkan ancaman yang serius kepada umum. Justeru, agen antibakteria baru dan effektif diperlukan untuk menangani situasi tersebut. Bakteria asid laktik (LAB) tidak patogenik boleh diisolasi daripada sayur-sayuran busuk dan ia berpotensi merencat aktiviti patogen bawaan makanan. Objektif kajian ini adalah untuk mengasingkan dan mengenalpasti LAB, juga untuk menentukan aktiviti perencatan bakteria terhadap patogen bawaan makanan. Sebanyak lapan isolat LAB telah diasingkan daripada tujuh jenis sayur-sayuran busuk. Aktiviti perencatan isolat LAB terhadap *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* L10 telah ditentukan dengan menggunakan kaedah peresapan agar-perigi. *E. coli* ATCC 8739 muncul sebagai organisma ujian yang paling mudah terdedah kepada isolat LAB yang diasingkan sebab zon perencatan semua isolat LAB terhadap *E. coli* ATCC 8739 ternyata lebih besar daripada organisma ujian lain. Agen antibakteria stabil dalam julat had nilai pH antara 2.0 sehingga 5.0. Aktiviti antibakteria tidak dapat diperhati apabila pH supernatan telah diselaraskan kepada pH 6.0 dan ke atas. Aktiviti antibakteria masih boleh diperhatikan walaupun telah terdedah kepada suhu yang tinggi dari 50^{o}C hingga 100^{o}C, namun zon perencatan menunjukkan trend penurunan dalam semua isolat terhadap bawaan makanan patogen apabila suhu meningkat.

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LIST OF ABBREVIATIONS

%	Percentage
µl	Microliter
µm	Micrometer
CFF	Cell-free filtrate
g	Gram
h	Hour
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
LAB	Lactic acid bacteria
M	Molarity
min	Minute
mL	Milliliter
mL/min	Milliliter per minute
mmol/L	Millimole per litre
MRS	de Man, Rogosa and Sharpe
NaOH	Sodium hydroxide
nm	Nanometer
^{o}C	Degree celcius
OD	Optical density
rpm	Rotation per minute
sp	Species

CHAPTER 1

INTRODUCTION

1.1 Introduction

Gram-positive, non-spore forming lactic acid bacteria (LAB) is well-known as probiotic strains and has been involved for long history in fermentations and food preservations. One of the significant properties of LAB that contributes to preservative effect is the capability of LAB to produce a variety of antimicrobial compounds through homo-fermentative or hetero-fermentative pathway (Abbott *et al*., 2009). The noteworthy antimicrobial compound is bacteriocin which is biologically active protein compounds produced in some LAB strains (Reis *et al*., 2012). Bacteriocin such as nisin has been used as food preservatives commercially. Moreover, organic acids such as lactic acid, acetic acid and propionic acid are responsible for the acidification of environment in which unfavorable for most of the spoilage and pathogenic microorganisms, thus contributes to bio-preservative. In addition, hydrogen peroxide and reuterin are also important metabolites of LAB that exert antimicrobial properties (Yang *et al*., 2012).

In the recent years, food borne diseases have shown a rising trend in Malaysia primarily due to the rapid population growth, consumption habits and hygienic issues (Sharifa Ezat *et al*., 2013). A serious threat poses to the public as some of the food borne bacteria showed antibacterial resistance such as the methicilin-resistant *Staphylococcus aureus*. Thus, a new and effective antibacterial agent through a convenient method should be studied to address the situation. The generally regarded as safe organism (GRAS), LAB is targeted because of its antibacterial potential against common food borne bacteria namely *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhii*, *Bacillus cereus*, *Shigella* sp., and *Listeria monocytogenes* (Kazemipoor *et al*., 2012; Senthilkumar *et al*., 2012; Galvez *et al*., 2010). Source and culture of LAB from fermented food, dairy product, meat, fresh fruits and vegetables are frequently published (Hajar & Hamid, 2013; Yusra *et al*., 2013; Yang *et al*, 2012; Oliveira *et al*., 2008; Trias *et al*., 2008). However, the micro-flora on rotten vegetable and the biological activities of LAB isolated from rotten vegetable are not well studied.

Rotten vegetables are often associated with microbial spoilage due to high polysaccharide cellulose, pectin and hemicellulose content. Their neutral pH as well as high water activity makes them an ideal niche for several bacteria (Barth *et al*., 2009; Doyle, 2007). Some of the normal flora of vegetables such as LAB is one of the contributors in rotting of vegetables (Dris & Jain, 2004). *Leuconostoc mesenteroides* had been found in decayed tomatoes and reported to cause sour rot type decay of tomatoes (Bartz *et al*., 1995). *Lactobacillus* sp. and *Leuconostoc* sp. also were observed in a complex segment in spoiled segment of fresh cut celery (Robbs *et al*., 1996). Thus, the possibility to isolate LAB from high availability, abundant and cheap rotten vegetables is relatively high and this makes the rotten vegetables a potential new source and culture for LAB.

1.2 Objectives

This study was carried out with the main objectives to investigate the antibacterial activities of LAB from rotten vegetables against food borne pathogens. The specific objectives of present study were:-

a) To isolate and identify the LAB from rotten vegetables
b) To characterize the antibacterial activities of the isolated LAB against food borne pathogens

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