

UNIVERSITI PUTRA MALAYSIA

DETECTION AND IDENTIFICATION OF LACTIC ACID BACTERIA ISOLATED FROM ROTTEN VEGETABLES

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DETECTION AND IDENTIFICATION OF LACTIC ACID BACTERIA ISOLATED FROM ROTTEN VEGETABLES



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ABSTRACT

Lactic Acid Bacteria (LAB) are the crucial bacteria that play important roles in the production of the fermented foods and also act as probiotic for the human when consume in adequate amounts of the bacteria. Some of the LAB also can caused harmful effects to human such as the species from the genus of *Weissella* sp. This research was performed in order to isolate and identify the LAB from the rotten vegetables. All of the isolated bacteria from Capsicum, Lettuce, Brinjal, Eggplant and Tomato were undergo gram stain, biochemical test, DNA purification and also gel electrophoresis. Most of the isolates inhabited by the LAB from the genus of *Lactobacillus* sp., only isolate form Capsicum 2 are from the genus of *Streptococcus* sp. All of the isolates were gram positive bacteria and the DNA extracted from all of the isolates were in size of less than 1.5 kb which were observed by using 1.5 kb ladder.

ABSTRAK

Lactic Acid Bakteria (LAB) adalah bakteria yang memainkan peranan yang penting dalam penghasilan makanan yang ditapai dan juga boleh bertindak sebagai probiotik kepada manusia apabila dimakan dalam jumlah yang berpatutan. Segelintir LAB juga boleh mengakibatkan kesan-kesan berbahaya kepada manus seperti spesies daripada genus *Weissella*. Kajian ini dijalankan untuk mengasingkan dan mengenalpasti LAB daripada sayuran rosak. Semua bakteria yang diasingkan adalah daripada sayur lada benggala, salad, terung, terung bulat dan tomato menjalani pewarnaan gram, ujian biokimia, penulenan DNA dan juga gel electrophoresis. Hampir kesemua bacteria yang diasingkan adalah daripada genus *Lactobacillus* sp., hanya bacteria yang diasingkan daripada Terung bulat 2 adalah daripada genus *Streptococcus* sp. Semua bakteria adalah pewarnaan gram yang positif dan DNA yang di ekstrak daripada semua bakteria adalah saiz yang kurang daripada 1.5 kb

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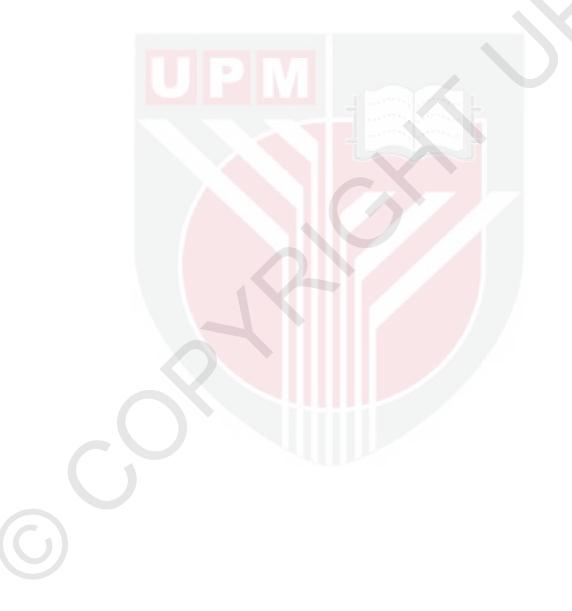
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LIST OF ABBREVIATIONS

sp.	=	Species
LAB	=	Lactic Acid Bacteria
%	=	Percentage
°C	=	Degree Celsius
kPa	=	kilo Pascal
rRNA	=	ribosomal ribonucleic acid
MRS	=	de-Mann, Rogossa and Sharpe
PCR	<u>_</u>	Polymerase Chain Reaction
TAE	2.7	Tris- Acetate- EDTA
kbp	-	kilo base pair
h	_	hour
ml	1	millilitre
rpm	_	rotation per minute
μl		microliter
ng	Ē	nanogram
V	=	Volt
TNTC	=	Too numerous too count
μm	=	micrometre
CO ₂	=	carbon dioxide
DNA	=	deoxyribonucleic acid
TE	=	Tris EDTA

CHAPTER 1

1. INTRODUCTION

Bacteria are the most widely organisms lived in this planet. They play a lot of important roles in everyday life. Some of the bacteria can degrade the dead organic material to be used by other organisms. And others can clean the contaminant of water and so on. Other than that, there are some bacteria that can give negative effect especially to their host. This type of bacteria known as pathogenic bacteria due to its characteristics. Generally pathogenic bacteria are harmful to another organisms in which they are the triggers to the cause of sickness among humans or animals. Some of the pathogenic bacteria are very virulent which can lead to the massive destruction of colony of humans or animals such *antrax* sp.. Besides that, there are also a good type of bacteria which are known as probiotic bacteria. This probiotic bacteria helps the host in so many ways such as digest the food consume by the host or helps the host to fight against the pathogen. For example is the *Lactobacillus* sp. in which they help to increase the efficiency in the digestion of food consumed by the host. Sometimes, these probiotic bacteria also can be pathogen which are known as opportunistic bacteria.

Lactic acid bacteria (LAB) are referring to the bacteria that can produce lactic acid whether as their major or minor end product. LAB are from the genera in the order of *Lactobacillales*, in which composed of *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus* and *Streptococcus*. LAB are among the most widely studied bacteria due to its characteristic which can prevent the spoilage of the food and also the development of pathogenic microorganisms (Babatunde, 2013). In addition, this LAB also can help to improve the texture and taste of the food such as cheese, yogurt, kefir and tempeh. LAB are gram positive bacteria, non-respiratory and also lack of catalase. LAB also non-spore forming bacteria and in-shaped of cocci, coccobacilli or rod. LAB can grow as aerobic, micro-aerobic or as facultative anaerobic microorganism.

LAB obtains their energy primarily from the metabolism of sugars by fermenting the glucose into lactic acid, carbon dioxide and ethanol. Due to their complex nutritional requirements, LAB required the environments in the present of sugars because of their limited biosynthetic ability (Barnali, 2010). Most of the LAB are non-pathogenic and free living or associated with the host as normal flora but some act as opportunistic pathogens which mostly are from the genus of *Streptococcus*.

1.1 OBJECTIVES

The objective of this project was:

- 1. To isolate Lactic Acid Bacteria (LAB) from the rotten vegetables
- 2. To identify the LAB at the genus level.

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