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**BACTERIOCIN – PRODUCING LACTIC ACID BACTERIA ISOLATED
FROM BUDU, FERMENTED CHILLI AND TEMPOYAK MADE OF
MALAYSIAN DURIAN MUSANG KING.**

By:

SHARIFAH KHAIRUNDALILA BINTI SAYED MILAM

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DECLARATION

I hereby declare that this thesis is based on my original work. I also declare the thesis has not previously or currently submitted by any other degree student at UiTM or other institutions.



SHARIFAH KHAIRUNDALILA BINTI SAYED MILAM

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ABSTRACT

Recently, there has been a concern on the natural preservatives in preserving foods. For this reason, many studies have been done in order to investigate and identify the microorganisms that can be used as biopreservatives, which are the lactic acid bacteria (LAB). LAB can produce bacteriocin that able to inhibit the growth of pathogenic organisms. However, this study was done due to lacking of studies on the LAB isolated from Malaysian traditional fermented foods such as *budu*, fermented chilli and *tempoyak* made of Malaysian Durian *Musang King*. Hence, the objective of this study is to screen the potential of the bacteriocin producing LAB from the traditional fermented food and also to study the effect of proteolytic enzymes towards them. The antimicrobial activity of the bacteriocin was tested against the selected indicator organisms which are *Staphylococcus aureus* (ATCC 43300), *Bacillus cereus* (ATCC 14579), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 13311) and *Listeria monocytogenes* (ATCC 7644). Four LAB isolated from *tempoyak* made of Malaysian Durian *Musang King* (*Lactobacillus plantarum* 1, *Leuconostoc mesenteroides ssp dextranicum* 2, *Pediococcus pentosaceus*, and *Lactobacillus plantarum* 1), two from fermented chilli (*Lactobacillus plantarum* 1, and *Lactobacillus fructivoran*) and one from *budu* (*Weissella cibaria*) were re-cultured from stock cultures stored at -20 °C and -40 °C, and the antimicrobial activity of the bacteriocin produced by the isolated LAB were tested against the selected indicator organisms by using disc diffusion method. The activity of bacteriocin is represented as the inhibition zone around the disc. Among the entire isolated LAB, LAB T6 (*Leuconostoc mesenteroides ssp dextranicum* 2) and LAB C3 (*Lactobacillus fructivoran*) were the best in showing inhibition activity against indicator organisms upon elimination of organic acid and hydrogen peroxide. The activity of bacteriocin towards the indicator organisms were inactivated after treated with proteolytic enzymes, the Proteinase K and trypsin. This finding indicates that, the bacteriocin produced by the isolated LAB does have the ability to inhibit pathogenic organisms and are safe for human consumption.

CHAPTER 1

INTRODUCTION

1.1. Background of the Study

Recently there has been a concern on the natural preservatives in preserving foods. For this reason, many studies have been done in order to investigate and identified the natural sources or microorganisms which could be used as the biopreservatives or the microorganisms that can act as the substances to avoid the spoilage of the food and extend its shelf life. One of the most widely being study is Lactic Acid Bacteria (LAB).

LAB is widely been used because of its status as generally recognized as safe (GRAS) (Arqués *et al.*, 2015). Under controlled conditions, they may also act as a starter cultures (Yang *et al.*, 2012). On top of that, according to Cintas *et al.*, 2001, (as cited in Yang *et al.*, 2012) LAB can produce substances such as organic acids, hydrogen peroxide and bacteriocins that are able to gives antimicrobial effects on the pathogenic bacteria. As for this study, bacteriocin is the substance that will be highlighted.

Bacteriocins are a peptide which is ribosomally synthesized, amphiphilic, heat-stable and most of them have a small size (about less than 10kDa). Bacteriocins may be used as natural preservatives in food industry (Zacharof & Lovitt, 2012). They can be divided into several groups based on their characteristics. In 1969, nisin is one of the types of bacteriocins that had been approved by the FAO/WHO Expert Committee on Food Additives as the food antimicrobial agent, this support the statement that the bacteriocins can destroy the membrane of the pathogenic cell and kill the sensitive microorganisms (Acuña *et al.*, 2012).

Up to now there are studies about the bacteriocins produced by LAB from *budu*, fermented chilli and *tempoyak* (Leisner *et al.*, 1999; Yuliana & Dizon, 2011; Yusra