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**ISOLATION AND CHARACTERIZATION OF *Lactobacillus* spp. ISOLATED
FROM BREAST MILK OF LACTATION MOTHER FOR PROBIOTIC
PROPERTIES**

By

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DECLARATION

I hereby declare that this thesis is my original work and has not been submitted previously or currently for any other degree at UiTM or any other institutions.

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ABSTRACT

Probiotics are live microorganism that can be implemented as a new approach to promote health. These organism are found to be an attractive means for health due to their probiotic properties including the ability to tolerate bile, low pH, and generate antimicrobial activity. Breast milk is a potential source of *Lactobacillus spp.* and one of the criteria that found beneficial is that it is human origin which could be more reliable sources. Lack of study on isolation of probiotic bacteria from breast milk were reported and some probiotic properties show a variation between strain from different region and population. Also, some of the probiotics which commercially used is not found to be a promising strain for all criteria of probiotic properties. This study was conducted to isolate, and characterize *Lactobacillus spp.* isolated from breast milk of lactating mother to be tested for two probiotic property tests include pH tolerance and antimicrobial activity. In this study, the isolates from breast milk expected to be *Lactobacillus spp.* were phenotypically and genotypically characterized. The isolates were initially identified by using API 50 CH kit. Further identification were executed by molecular characterization involving PCR amplification, sequencing and phylogenetic analysis. The isolates were then subjected to probiotic test include pH tolerance and antimicrobial activity. Four isolates were determined as Gram positive bacilli, catalase negative with no endospore formation. Identification by API 50 CH kit revealed the isolates as *Lactobacillus fermentum*. PCR amplification results in separation range from 1500 bp to 2000 bp. Sequencing and phylogenetic analysis done with reference strains retrieved from National Center for Biotechnological Information demonstrated showed strong relationship between Isolates 1 and 3 with the reference strains. Among the isolates, Isolate 3 and Isolate 4 show slightly greater tolerance to acid after 5 hours. The differences found in the growth rate were not significant ($p > 0.05$). Test for antimicrobial activity show a significant result for non-neutralized supernatant, ($p < 0.05$) and Isolate 1 and Isolate 2 show greater antimicrobial activity against the pathogenic organisms tested. Not much inhibition observed for antimicrobial activity using neutralized supernatant. Due to the ability to tolerate pH and antimicrobial activity observed, the isolates were considerable to be subjected for further test to find the best strain to be regarded as the best candidate for probiotic.

Keywords : *Lactobacillus*, breast milk, probiotics, molecular characterization, antimicrobial

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Probiotics are live microorganisms which confer benefit to the host when administered in sufficient amounts (Anandharaj & Sivasankari, 2014). Lactic acid bacteria are considered as probiotics as they are capable in promoting health and nutritional benefit (Pisano *et al.*, 2014). *Lactobacillus spp.* is one of probiotic bacteria which belong to lactic acid bacteria (LAB)(Klein, Pack, Bonaparte, & Reuter, 1998). According to Shokryazdan *et al.* (2014), lactic acid bacteria, particularly *Lactobacillus*, are the most frequently used microorganisms as probiotics due to the consideration that they are required constituent of the gut microflora and also these bacteria have “Generally Recognized As Safe” (GRAS) status and certain species obtained qualified presumption of safety (Pisano *et al.*, 2014). However, researchers also claim that the rising concern in probiotics has resulted in many putative probiotics products sold without sufficient study on probiotic properties of the strains which can cause unreliable efficacy of the products (Shokryazdan *et al.*, 2014).

FAO/WHO have proposed guidelines which recommend that identification of all potential probiotics are supposed to be correctly done using both of phenotypic and genotypic methods with various test followed on investigation of its survival ability and functional properties. (Shokryazdan *et al.*,2014 ; FAO/WHO, 2002).