UNIVERSITI TEKNOLOGI MARA

CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM MILK AND CURD AND ITS IDENTIFICATION USING AMPLIFICATON OF 16S rRNA GENE

NUR SHAHEDAH BINTI ISMAIL

Project submitted in fulfilment of the requirements for the degree of

Bachelor of Medical Laboratory Technology (Hons.)

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DECLARATION BY STUDENT

Project entitled "Characterization of Lactic Acid Bacteria Isolated from Milk and Curd and Its Identification Using Amplification of 16S rRNA Gene" is a presentation of my original research work. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to literature, and acknowledgement of collaborative research and discussions. The project was done under the guidance of Project Supervisor, Dr. Maimunah Binti Mustakim and Cosupervisor, Pn. Rozila Binti Alias from University Selangor. It has been submitted to the Faculty of Health Sciences in partial fulfilment of the requirement for the Degree in Bachelor of Medical Laboratory Technology (Hons).

Student's signature:

(Nur Shahedah binti Ismail)

2015679602

930811-10-5326

Date: _____

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ABSTRACT

In recent years, there are increasing numbers of food industry emphasizing the use of selected lactic acid bacteria (LAB) supplemented in raw and fermented milk. The industry become important contributors in producing and developing a beneficial food that confer human health. The aim of this project is to identify and characterize the LAB isolated from local dairy products. The bacterial isolates were identified and characterize based on the morphological study and biochemical tests. Genotyping identification was done using PCR amplification of 16s rRNA gene. Potential probiotic properties were screened by antibiotic susceptibility test and bile salt tolerance tests. A total of 11 isolates were obtained which three isolates were from fermented milk (curd) and the remaining eight isolates were obtained from goat's milk. Morphological study showed isolates #1 to #6 and #11 were rod shaped while isolates #7 to #10 were spherical shaped bacteria. All the isolates were gram positive and they exhibited negative for catalase and oxidase. Molecular identification of 16S rRNA gene using forward primer (*pbl16*) and reverse primer (*mlb16*) showed amplicons at 540 base pair. Probiotic screening test showed all isolates were able to grow in $0.3 \,\% \,(\text{w/v})$ bile and the coefficient of growth inhibition were less than 0.5, which considered as bile tolerance. The antibiotic susceptibility test indicated that all of the isolates were resistant to Vancomycin. In conclusion, the LAB in the milk and curd were successfully identified and characterized using morphological studies, biochemical tests and molecular technique.

Keyword: Lactic acid bacteria, milk, 16S rRNA gene amplification, bile tolerance, probiotic.