**UNIVERSITI TEKNOLOGI MARA**

# THE USE OF MOLECULAR TOOLS IN IDENTIFYING POTENTIALLY PROBIOTIC *Lactobacillus spp*. ISOLATED FROM COW'S AND GOAT'S MILK

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# DECLARATION

I hereby declare that the work presented in this thesis has been written entirely by myself except for quotations and summaries where stated otherwise by reference and acknowledgement. This thesis also has not been submitted previously in whole or in part for any other degree in UiTM or any other universities.

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# ABSTRACT

The interest in consuming products which contained probiotic especially *Lactobacillus spp*. are at increasing trend since they have been proved to offer numbers of health benefit on human. At present, *Lactobacillus spp.* are known to exist in many different sources but studies on those sources remain limited in Malaysia. Hence, investigation on two sources of potentially probiotic lactobacilli which are cow's and goat's milk obtained from local farms in Malaysia was conducted. This research focused on isolating and identifying potentially probiotic *Lactobacillus spp*. from cow's and goat's milk using both phenotypic and genotypic characterization. Initially, isolates from both samples were selected by phenotypic identification and further identified by molecular technique. DNA of isolates were extracted using NucleoSpin® Microbial DNA kit. Next, PCR amplification of 16S rRNA gene was performed using forward primer, 5' - GCT GGA TCA CCT CCT TTC - 3' and reverse primer, 5' - CCT TTC CCT CAC GGT ACT G - 3'. Amplicons were detected through 1.2 % agarose gel electrophoresis and visualized using ImageQuant$^{TM}$ LAS 500. Bile salt tolerance was then performed to screen for probiotic property of six chosen isolates and their safety profiling was conducted through antibiotic susceptibility testing (AST). A total of 11 isolates revealed to be Gram-positive rod, negative for catalase and oxidase test were subjected to molecular identification. Only six isolates (CM1, CM2, CM3, GM1, GM2, GM3) belong to *Lactobacillus spp*. with two sizes of amplicons which are 710 bp and 920 bp. All six isolates were considered to be bile tolerant with more than 50 % survival in 0. 3 % bile salt and their AST results displayed resistance towards sulfamethoxazole/trimethoprim, gentamicin and vancomycin. In conclusion, this research successfully identified six isolates of potentially probiotics *Lactobacillus spp*. from cow's and goat's milk.

**Keywords:** *Lactobacillus*, probiotic, milk, molecular identification, bile salt tolerance