

**UNIVERSITI TEKNOLOGI MARA**

**SCREENING AND  
CHARACTERISATION OF LACTIC  
ACID BACTERIA ISOLATES AS  
POTENTIAL PROBIOTIC FROM  
DIFFERENT STAGES OF SOYBEAN  
TEMPE PROCESSING**

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## ABSTRACT

Nowadays, health consciousnesses among the consumers are increasing. The market of healthy food like ready fermented food is in demand especially ready fermented food with probiotic as they commonly contain lactic acid bacteria (LAB). However, the information of LAB presence during the processing stages of fermented food is still scarce. Therefore, isolation of LAB from tempe was carried out at different stages of tempe processing to examine the occurrence of LAB and to identify the isolates with the most promising potential as probiotic. Isolation was done using conventional plating method while morphological, physiological and chemical characteristics with the use of API 20 Strep, API 50 CHL kit and 16S rRNA gene sequences were employed to identify the LAB isolates. Acid (pH 1.5, 2.0, 4.0, 6.5 and unmodified broth) and bile (0.3%) tolerance along with antimicrobial activity tests were conducted to screen for probiotic characteristics. A total of 14 LAB isolates were obtained from all stages. Identification of all LAB isolates using API kits revealed isolates A, B, C, D, E, F and I as *Enterococcus faecium*, isolates G, H, M and N as *Leuconostoc mesenteroides* and isolate J as *Lactobacillus plantarum*. Isolate K and L however were unable to be identified. Molecular identification using 16S rRNA had revealed that isolate G (*Leuconostoc mesenteroides*) and J (*Lactobacillus plantarum*) were re-identified as *Leuconostoc lactis* and *Leuconostoc* spp. respectively. Whereas, two previously unidentified isolates were identified as *Alicyclobacillus* spp. Characterisation using acid and bile tolerance tests, showed that all LAB isolates responded differently as they were strain dependent. Isolates C, D and E (*Enterococcus faecium*) were resistant at pH 1.5, 2 and 4 up to 2 h exposure thus were selected as the best strain that can survive the strict acidic conditions of the stomach. As for bile salt tolerance test, isolates B, D and E (*Enterococcus faecium*) were selected as the best strain since they remained resistant up to 4 h of such exposure. Other LAB isolates appeared to be tolerant to both acid and bile salt with exceptions of isolates K and L. Meanwhile, antimicrobial activity of all 14 LAB isolates showed good antibacterial activity against common pathogenic microorganisms, *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*, with high and medium antimicrobial activity. However, no activity was observed when tested against *Candida albicans* and *Aspergillus niger*. Overall, LAB isolates were present at all stages of tempe processing with LAB isolates B, C, D and E showed the most promising strains as probiotic. Further investigations were needed to fulfil all the other probiotic criteria not tested in this study. The unique characteristics of these LAB strains offer their potential towards food and pharmaceutical industries.

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