CHAPTER 8

CONCLUSION AND RECOMMENDATIONS

8.1 Conclusion

The overall aim of this project was to isolate, characterise and identify potential probiotic lactic acid bacteria (LAB) from Malaysian fermented shrimp and fish, as well as to evaluate their contribution towards cholesterol modulation. In terms of nutrient content, bosou is the most competent candidate to provide good nutrients with low salt content. A total of 59 LAB strains isolated from belacan, bosou and non-cooked budu were confirmed as Gram-positive and catalase-negative. However, only seventeen isolates could tolerate acidic environments, indicating their ability to survive in the stomach. Interestingly, all seventeen isolates were tolerant to bile, not exhibiting β-hemolysis on blood agar, demonstrating considerable antagonistic activities against common food-borne pathogenic bacteria and displaying different resistance towards multiple antibiotics. In addition, the isolates also displayed considerable hydrophobicity and autoaggregation ability.

Using a molecular approach, seven isolates were identified as *Lactiplantibacillus plantarum*, while the remaining were identified as *Lacticaseibacillus paracasei*. All of the isolates did not exhibit BSH activity but possessed varying degrees of cholesterol removal capabilities from the growth medium. *L. plantarum* strain BE7, *L. plantarum* strain BO1 and *L. paracasei* strain BUM6 were chosen to be further characterised for their probiotic properties and cholesterol modulation based on principal component

analysis. The isolates could adhere successively to the HT-29 cells, the intestinal cell model, without causing cytotoxicity. Additionally, the transcriptomic profiles of NPC1L1, CD36, SCARB1 and NR1H3 genes expressed by HT-29 cells when treated with these three strains also showed the potential reduction of cholesterol absorption into the enterocytes, proving their prospective as probiotics for the food industry and alternative treatment in the management of hypercholesterolemia.

8.2 Recommendations

The current study highly recommends the use of *L. plantarum* strain BE7, *L. plantarum* strain BO1 and *L. paracasei* strain BUM6 as potential probiotics. However, cholesterol modulation in the intestinal environment not only involves proteins that transport cholesterol into the cell but also proteins that regulate cholesterol efflux. Thus, further work could involve an analysis of the effect of the strains upon gene expression of these transport proteins, for example, ATP-binding cassette transporters G5 (ABCG5) and ATP-binding cassette transporters G8 (ABCG8).

In addition, this study focused on the ability of probiotics to regulate the expression of genes related to the regulation of cholesterol in cell-line models. Further work could be done in-vivo to assess whether the isolates can exhibit similar abilities in animal models. The investigation into the interaction of these LAB strains with the normal microbiota in the intestine must also be conducted to understand the combined effects of their presence in the same environment. Some extension could also be done to observe the relationship between dietary cholesterol supplied to the animals, the expression of genes involved in modulation of cholesterol and their total cholesterol level.

LAB strains with probiotic characteristics and cholesterol modulation properties should be recommended for use in food formulations. Further study could also involve the investigation of isolates' stability on food formulation parameters, before they could be developed as commercial products with excellent functions that are suitable for various groups.

Additionally, since *L. plantarum* strain BE7, *L. plantarum* strain BO1 and *L. paracasei* strain BUM6 were previously isolated from the fermented food that could be taken raw, research could be done to investigate the ability of these isolates to be used as a novel starter culture in the development of the same fermented food. Moreover, their stability in the final products and the ability to retain the probiotic characteristics should also be assessed.