CHAPTER 5

CONCLUSIONS

5.0 Conclusions

5.1 Conclusion

Indigenous lactic acid bacteria could be isolated from local foodstuffs. In order to isolate these bacteria properly, the choice of isolation media would be critical. In this case, MRS media was chosen to fulfil the needs of the lactic acid bacteria. Lactic acid bacteria are fastidious by nature, as they require a substantial amount of polypeptides, amino acids, B class vitamins and some essential minerals for growth.

Several types of sugars were used with MRS media in isolating the lactic acid bacteria. 126 bacterial isolates were obtained from four local food sources viz, tapai, tempoyak, chili boh and fresh goat's milk. Catalase test and Gram's staining were employed to differentiate lactic acid bacteria from the rest. Out of 126 isolates, 55 were identified as lactic acid bacteria i.e. they were gram-positive and catalase negative. They were further screened to distinguish between the homofermentative and heterofermentative isolates using the gel plug test.

Sixteen isolates were identified as homofermenters. Seven of these isolates were put through a series of environmental conditions to further screen and select potential isolates. These series of tests were done to determine the suitability of the isolates in the process of producing lactic acid for the purpose of making PLA. Tsai and co-workers (1995) have notes several criteria in choosing lactic acid bacteria strains for industrial lactic acid fermentation. These criteria are the ability to grow on glucose, homofermentative, able to produce the desire optical isomer(s) of lactic acid, resistant to product inhibition and the ability to grow at high temperature to minimise microbial contamination.

Three of the seven isolates were chosen on the basis of their ability to produce single lactic acid isomer, tolerance to high temperature, product inhibition, osmotolerance

and tolerate variable pH. Of these three isolates, TapLac seems to be a good candidate for the production of lactic acid for the purpose of making poly(lactic acid). On comparison when cultivated at various temperatures, TapLac displayed good lactic acid production profiles between 37°C and 45°C. Although FM, seems to tolerate high temperature, but the lactic acid production profile is less superior to TapLac.

The threshold pH plays an important role choosing the appropriate isolate. An isolate that is able to tolerate low pH could theoretically produce more lactic acid than an isolate that can not tolerate low pH. By looking at the biomass and lactic acid production profiles at cultivated at various initial pHs, TapLac seems to display better biomass and lactic acid production profiles at acidic and neutral pH to TapSuc and FM.

One other aspect that makes TapLac a prime candidate between the three chosen isolates was the ability of TapLac to tolerate a certain amount of high NaCl concentrations. This is in relation to the loss of water to outside and the cell and a shift in osmotic pressure and potential loss of the turgor pressure by the cells. Although FM seems to tolerate a higher salt concentration compared to Taplac and TapSuc, the amount of lactic acid produced by this isolate was very low. Also the need to bear in mind that, in the process of lactic acid production, the salt concentration increases over time as more acids are produced and neutralised into salts. TapLac otherwise would be able to tolerate a certain amount of NaCl concentration, better than TapSuc and comparable to FM at certain higher NaCl concentration. An overview of the studies conducted in this project is presented in Figure 5.1.

Recommendation for future work, should involves studies on alternative media for the cultivation of lactic acid bacteria, preferably from industrial waste such food processing waste which contains high polypeptone and amino acids. Furthermore, alternative carbon

sources for the production of lactic acid need to be explored, as the market price of pure glucose is high. This will help to lower the cost of producing lactic acid.

PART IV

Further Physiological Studies

Three of the isolates were chosen based on their performances at different environmental conditions after 48 hours of cultivation. End point pH, biomass, glucose consumption and lactic acid production were determined.

- Different cultivation temperatures 15°C, 30°C, 37°C, 45°C, 50°C → Figures 4.1 to 4.5
- Different initial pH 4.5, 7.0, 9.0 → Figures 4.6 to 4.10
- Different NaCl concentrations (w/v) 1.5%, 2.5%, 5.0%, 7.5%, 10.0% → Figures 4.11 to 4.15

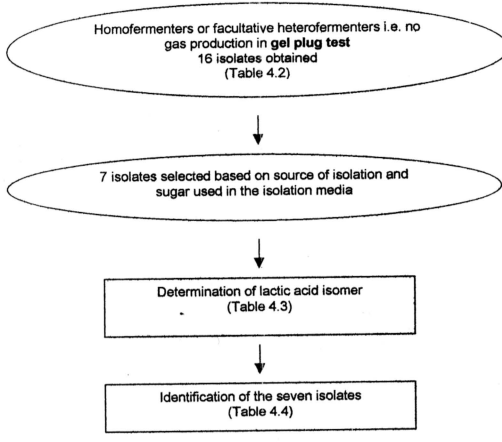
PART V

Time-Course Studies Using Shake Flasks

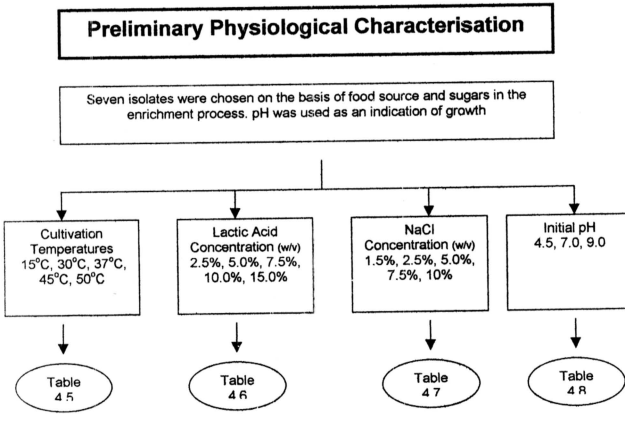
End point pH, biomass production, glucose consumption and lactic acid production profiles over 54 hours were determined

→ Figures 4.16 to 4.20

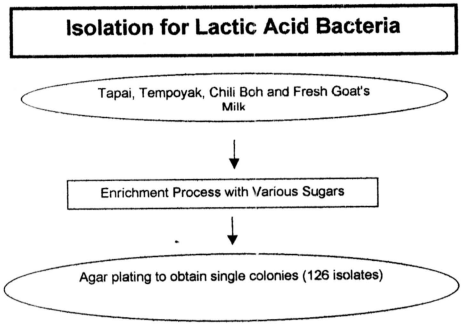
Figure 5.1: A schematic overview of the processes involved in isolating, screening, characterisation and determining potential lactic acid bacterial isolates for industrial lactic acid production


Homofermenters or facultative heterofermenters i.e. no gas production in **gel plug test**
16 isolates obtained
(Table 4.2)
7 isolates selected based on source of isolation and sugar used in the isolation media
Determination of lactic acid isomer
(Table 4.3)
Identification of the seven isolates
(Table 4.4)

PART III


Preliminary Physiological Characterisation
Seven isolates were chosen on the basis of food source and sugars in the enrichment process. pH was used as an indication of growth
Cultivation Temperatures
15°C, 30°C, 37°C, 45°C, 50°C
Lactic Acid Concentration (w/v)
2.5%, 5.0%, 7.5%, 10.0%, 15.0%
NaCl Concentration (w/v)
1.5%, 2.5%, 5.0%, 7.5%, 10%
Initial pH
4.5, 7.0, 9.0
Table 4.5
Table 4.6
Table 4.7
Table 4.8

PART I



PART II