

## CHAPTER V

### CONCLUSIONS

1. The optimum condition for glucansucrase production from a thermotolerant *Bacillus licheniformis* TH4-2 was 42 hours of cultivation in 5 % soluble starch from cassava supplemented with 0.4% ovalbumin , pH 6.5 at 45°C.
2. Glucansucrase was 112 fold purified with a 28% yield. The enzyme had molecular mass of 64 kDa, optimum pH and temperature were 6.0 and 45°C. The apparent  $K_m$  and  $V_{max}$  values for sucrose substrate were 38.14 mM and 0.042  $\mu\text{mole}/\text{min}$ , respectively , and The apparent  $K_m$  and  $V_{max}$  values for melibiose acceptor were 148 mM and 0.0072  $\mu\text{mole}/\text{min}$ , respectively.
3. The enzyme was able to synthesize prebiotic oligosaccharides from various saccharides. G1 could not act as acceptor. Melibiose was one of the best glucosyl acceptors.
4. The optimum condition for OS production was 15% (w/v) melibiose ( acceptor ), 5% (w/v) sucrose ( donor ) , glucansucrase concentration of 5U/ml at pH 6.0 , 45°C for 24 hours.
5. After optimization, two products were obtained from melibiose acceptor, main product (product A at Rt 8.3 min) and minor product (product B at Rt 10.3 min) with the yields of 17.2% and 3.3%, respectively.

6. The structural identification of the main product revealed the trisaccharide structure of 504 daltons of melibiose linked by an  $\alpha$ -1,6 bond with one molecule of glucose.
  
7. The main product (product A at Rt 8.3 min) can support significant growth of *Lactobacillus acidophilus* but at a lower rate than melibiose and FOS (commercial). Raffinose, on the contrary, did not support growth of this bacteria.