CHAPTER V

CONCLUSIONS

1. Recombinant colonies with CGTase gene were obtained from *Paenibacillus* sp. RB01 and *Paenibacillus* sp.T16 and named as pRB and pT, respectively
2. Both CGTase genes from the transformants contained the open reading frame of 2194 nucleotides coding for 732 amino acid residues for pRB and 2139 nucleotides coding for 713 amino acid residues for pT. A putative promoter and a Shine-Dalgarno sequence (SD) could be located upstream of the gene.
3. The deduced amino acid sequence of pRB and pT showed 97% and 99% homology with the CGTase of *Paenibacillus* sp. A11, respectively.
4. The CGTase from pRB was purified to homogeneity by starch adsorption and followed by DEAE-cellulose column chromatography with final yield of 7% and 26 purification folds purification with specific activity of 14,688 units/mg protein.
5. The CGTase from pT was purified to homogeneity by starch adsorption and Phenyl-sepharose column chromatography to final yield of 44% and 93 purification folds with specific activity of 89,667 units/mg protein.
6. The molecular weight of 65 kDa and 77 kDa were estimated for enzymes expressed from pRB and pT respectively, using SDS-PAGE.
7. The major band of isoelectric point was 5.85 for pRB and pT.
8. The optimum pH and temperature for dextrinizing activity of RB01 and pRB were pH 5-6, 60°C and pH 5-9, 50-70°C. The optimum pH and temperature for cyclization activity of RB and pRB were pH 6.5, 60-70°C and pH 6.5, 50-60°C.

9. The optimum pH and temperature for dextrinizing activity of T16 and pT were pH 5-9, 60°C and pH 5-9, 40-70 °C. The optimum pH and temperature for cyclization activity of T16 and pT were pH 6.5, 60-70°C and pH 6.5, 50-70°C.
10. All four CGTases (from RB01, pRB, T16 and pT) were stable in a wide pH range of 6-10 and temperature at 40-60°C within 30 minutes. They were stable at pH 7-9 and temperature of 45-55 °C within 60 min.
11. Thermostability at 70°C of cyclization activity was improved when 6% soluble starch was present in both wild types and transformants.
12. The CGTase had high specificity towards cyclic-oligosaccharides and appropriate size linear oligosaccharides, comprising of at least three glucose units linked by the α-1,4 glycocsidic bond.
13. The turnover numbers (k_{cat}) of the CGTase from both RB01 and pRB were higher for G-α-CD than those of β-CD and its derivatives. The turnover numbers (k_{cat}) of the CGTase from T16 and pT with natural CD (α-CD and γ-CD) were higher than those of β-CD and its derivatives. The efficiency (k_{cat}/K_m) of CGTases from both RB01 and pRB were highest with HP-β-CD and the efficiency (k_{cat}/K_m) of CGTase from T16 and pT were highest with β-CD. Transformant CGTases showed lower K_m for β-CD and its derivatives.
14. The dominant end-products obtained from RB, pRB and pT were β-CDs, while T16 gave nearly equal amount of all CDs with slightly higher amount of γ– CDs.
15. The enzyme catalyzed the conversion of starch to CDs with a ratio of α– : β– : γ–CD of 0.57 : 1 : 0.13 for RB01 and 0.21 : 1 : 0.05 for pRB and 0.83 : 1 : 1.1 for T16 and 0.25 : 1 : 0.51 for pT.
16. pRB and pT required shorter time (1/3) incubation and produced the CGTase with the high specific dextrinizing activity than their original cells.