CHAPTER V

CONCLUSION

1. CGTase from *Bacillus* sp. A11 was purified approximately 44.8 folds with a 30% yield and specific activity of 5,000 units/mg protein.
2. The amino acid composition of CGTase showed that 60 mol% of the content was polar amino acids. Acidic amino acids: aspartic acid, asparagine, glutamic acid, and glutamine was rather high (25 mol%).
3. N-terminal amino acid sequence up to 20 th residues of *Bacillus* sp. A11 was found to be APDTSNYNKQNFKTDVIYQI, which was 60-80 % in homology to other CGTases.
4. Two synthetic oligonucleotides of 17 bases, PNB and PCC were designed from translation of N- and C-terminal amino acid sequences of CGTase. PNB was 5′ HO-CAACAAGCA(G/C)AATTTCC-OH 3′ and PCC 5′ was HO- GTA(C/T)TATGATGTCAGCG-OH 3′. They were used to detect transformant cells containing CGTase gene by colony hybridization.
5. 360 positive clones showed signal with PNB and PCC and only five CGTase-producing transformants (14, 247, 276, 885, and 909) were screened and isolated by starch hydrolyzing activity on LB-starch agar plate, dextrinizing activity, PICT, and CD-TCE activity.
6. Transformant 909 gave the highest CGTase activity but activity was lower than *Bacillus* sp. A11.

7. Localization of CGTase in transformant 909 was determined and 87.5% was found in extracellular fraction. For wild type, 100% of the activity was expressed as extracellular enzyme.
8. CGTase of transformant 909 yielded α- : β- : γ-CD product ratio of 1: 4.6: 1.6.
9. Crude CGTase of transformant 909 showed the same electrophoretic patterns as that of *Bacillus* sp.A11 when analyzed by non-denaturing and SDS-PAGE.