CHAPTER VI

CONCLUSIONS

1) Alpha – glucosidase (*AG*) expression is highest in forager bee. Quantitative analysis by RT – PCR indicates that an expression profile of *AG* gets increased in nurse bee (386.633) and forager bee (760.589), respectively. But AG is not express in egg (16.082).

2) The cDNA sequence of *AG* (1,739 bp) was obtained by RT – PCR. In addition, the deduced amino acid sequence of AG (568 amino acids) was obtained.

3) By blast, alignment of *AG* sequence in *A. florea* with related genes in other organisms presents the highest similarity of 95% to that in *A. mellifera.*

4) Phylogenetic trees of amino acid sequence by UPGMA and Neighbor – Joining were constructed. The result also supports that the *AG* from *A. florea* was closed to *AG* in *A. mellifera.*

5) The suitable purification procedure of AG in *A. florea* was chromatographied on DEAE – cellulose (0.171 u/ mg), Superdex 200 (2.385 u/ mg), and concentrated by centrifugal filter at MWCO 10,000 Da (4.043 u/ mg). This procedure provides the highest activity.

6) The specific activity of AG was obtained from DEAE – cellulose at 0.171 u/ mg (with 95 % ammonium sulfate, AS, saturation) and at 4.5 u/ mg (without AS precipitation). It is possible that AS causes the loss of AG activity.

7) From Sephadex G – 150 protein in active fractions was separated by SDS – PAGE and renatured. The activity band of AG (93 kDa) could be recovered.

8) The most active fraction from Superdex 200 was concentrated and separated by SDS – PAGE. After the plot of R_f value and log MW, the MW of candidate AG was about 73 kDa. Then, the band was excised and sequenced by MALDI - TOF MS.

9) MALDI - TOF peptide mass maps of purified AG showed six matching masses of AG in *A. mellifera* (Q17058), score 70, with 12% coverage (based on the M_r of 65523 Da).

10) The optimum condition for partial purified AG was at pH 5, at temperature of 55°C, at incubation time of 40 min, and in sucrose concentration of 80 mM.