CHAPTER IV

CONCLUSIONS

1. The phenylalanine dehydrogenase (*phedh*) gene from *Bacillus lentus* was successfully cloned into an expression vector, pRSFDuet-1 resulting in recombinant plasmid pPheDH and expressed in *E. coli* BL21(DE3). The recombinant clones showed different levels of the specific activity in the range of 35.1 to 71.7 units/mg protein. The highest specific activity was 71.7-fold higher than that of *B. lentus*.

2. Each of the seven genes (*aroB* encoding 3-dehydroquinate synthase, *aroL* encoding shikimate kinase II, *glpF* encoding glycerol facilitator, *glpK* encoding glycerol kinase, *pheA* encoding chorismate mutase/ prephenate dehydratase, *tktA* encoding transketolase and *yddG* encoding aromatic amino acid exporter) was cloned into pRSFDuet-1 vector leading to recombinant plasmids pAroB, pAroL, pGlpF, pGlpFK, pPheA, pTktA and pYddG. These plasmids were separately propagated in *E. coli* BL21(DE3) to be used as source of the gene preceded by T7*lac* promoter for construction of different gene combinations.

3. After culturing each *E. coli* BL21 (DE3) harboring pAroB, pAroL, pGlpF, pGlpFK, pPheA, pPheDH, pTktA and pYddG in LB medium supplemented with 1 mM IPTG for 3 h, the six proteins (AroB, AroL, GlpK, PheA, PheDH and TktA) were locally synthesized in cytoplasm and their amounts were sufficient to be detected on Coomassie Brilliant Blue stained SDS-PAGE gel. In contrast, the synthesized YddG and GlpF proteins were embedded in cell membrane and their amounts in membrane fractions were insufficient to be detected on gel stained with Coomassie Blue.

4. The different combinations of various genes together with *phedh* were created in a single pRSFDuet-1 vector resulting in nine recombinant plasmids pPY, pPYF, pPYFK, pPTY, pPTFY, pPTFKY, pPTFBY, pPTFBLY and pPTFBLYA. These plasmids were introduced to *E. coli* BL21(DE3) to compare their L-Phe production to that of pPheDH containing *phedh* alone.

5. The multiple genes cloned in a single vector pRSFDuet-1 could be simultaneously expressed in recombinant *E. coli* when monitoring by SDS-PAGE.

6. The production medium containing 30 g/L of glycerol and 50 g/L of $(NH_4)_2SO_4$ was optimum medium for *E. coli* BL21(DE3) containing pPheDH.

7. The different concentrations of the IPTG inducer (0.25, 0.5 and 1.0 mM) had no effect on growth, consumption of glycerol and production of L-Phe of all recombinant clones so long as the concentration was at least 0.25 mM.

8. Glycerol was confirmed as the best carbon source for L-Phe production by constructed *E. coli* BL21(DE3) when compared to glucose.

9. The maximum L-Phe production rates of the induced pPY, pPYF and pPYFK clones were 1.4-, 1.8- and 1.3-fold higher than the maximum production rate of the pPheDH clone. An aromatic amino acid exporter can promote L-Phe production rate of *E. coli* containing *phedh*. Also, glycerol facilitator showed little effect on the production rate when its gene was co-expressed with *phedh* and *yddG*. In contrast, the expression tandem of glycerol facilitator and glycerol kinase could not increase the production rate of L-Phe from *E. coli* containing *phedh* and *yddG* genes.

10. The six different clones (pPTY, pPTFY, pPTFKY, pPTFBY, pPYFBLY and pPTFBLYA) had the production rates of 2.21-3.36 mg/L h with final values of L-Phe concentrations of 340-429 mg/L which were 1.4- to 2.1-fold higher than rate of pPheDH. The pPTFBLY clone showed the highest in both L-Phe production rate and final concentration of L-Phe without induction by IPTG.

11. For pPYF clone, the peak L-Phe concentration in the bioreactor (545 mg/L) was nearly 2.0-fold the peak value obtained in the shake flask (280 mg/L). Thus, L-Phe productivity of the bioreactor was nearly 3.8-fold greater than the shake flask productivity. A better supply of oxygen in the bioreactor explained its higher productivity compared to the shake flask.