CHAPTER V

CONCLUSION

1. Three peptide fragments of phenylalanine dehydrogenase from *Bacillus lentus* obtained by the lysyl endopeptidase digestion were isolated. Their sequences were AVIIGDPQKD, AIAGSANFQLLTEDHGRQLAD, and GILYAPDYIVNSGGLIQV ADELYEVN. These obtained amino acid sequences were then used for degenerated primer design for phenylalanine dehydrogenase gene sequencing and cloning.

2. The full length phenylalanine dehydrogenase gene from *Bacillus lentus* was sequenced by inverse PCR and successfully cloned into *E. coli* BL21(DE3) using the expression vector, pET-17b. This gene has a large single open reading frame of 1,143 nucleotides encoding the polypeptide of 380 amino acid residues.

3. The percentage of similarity of nucleotide sequences of phenylalanine dehydrogenase from *Bacillus lentus* to those of *Bacillus badius*, *Sporosarcina ureae*, *Bacillus sphaericus*, *Thermoactinomyces intermedius* and *Rhodococcus* sp. M4 were 99, 65, 62, 57 and 10%, respectively.

4. Comparison of the deduced amino acid sequences of phenylalanine dehydrogenase from *Bacillus lentus* with those in the EMBL-GenBank-DDBL database showed 99, 67, 66, 62, 53 and 32% similarity to those of *Bacillus badius, Bacillus sphaericus*, *Sporosarcina ureae*, *Bacillus halodurans*, *Thermoactinomyces intermedius* and *Rhodococcus* sp. M4, respectively.

5. The recombinant clones exhibited various levels of the specific activity from 9.34-92.0 units/mg protein. Moreover, the highest specific activity was 92.0 fold higher than that of *Bacillus lentus*.

6. With regard to induction with 0.2 mM IPTG for 8 hours, *phedh* gene showed the highest expression.

7. Despite the fact that the recombinant clone (pBLpheDH) was daily subcultured for 50 days, the *phedh* gene expression in *E. coli* BL21(DE3) remained 57.7% of that of the parent. It was implied that the recombinant clone has high stability of recombinant plasmid.

8. PheDH from pBLPheDH clone was purified 2.11 fold with a 31.0% yield by procedure involving 40-50% saturated ammonium sulfate precipitation, DEAE-Toyopearl and Butyl-Toyopearl column chromatography.

9. The molecular weight of the native was determined to be about 340,000 Da and consisted of 8 identical subunits (approximately 42,000 Da).

10. The enzyme had high substrate specificity on L-phenylalanine in the oxidative deamination and phenylpyruvate on the reductive amination.

11. The enzyme required NAD^+ as a natural coenzyme for oxidative deamination. $NADP^+$ was inert whilst the NAD^+ analog, 3-acetylpyridine-NAD^+, showed 1.67 times higher activity than NAD^+.

12. The optimum pH of the enzyme for the oxidative deamination and reductive amination were 10.7 and 7.8, respectively.

13. The optimum temperature of the enzyme for the oxidative deamination and reductive amination were 45°C and 50°C, respectively.

14. The enzyme was stable over a pH range from 7.0 to 11.0.

15. The enzyme was fully stable at 45°C for 1 hour and retained 50% of its activity after incubation at same temperature for 3 days.

16. The apparent K_m for L-phenylalanine, NAD^+, phenylpyruvate, ammonium and NADH were 0.45, 0.40, 0.15, 48 and 0.15 mM, respectively.