# CHAPTER V

## CONCLUSION

1. The bacteria strain K-1, screened from soil samples which produced Lys 6-DH was identified as *Achromobacter denitrificans*.

2. Lys 6-DH from *A. denitrificans* was purified to175 fold with a 34% yield by procedure involving 0-60% ammonium sulfate precipitation, DEAE-Toyopearl 650 M, 0-40% ammonium sulfate precipitation, Sephadex G-150, Mono Q HR 10/10 and Phenyl Sepharose XK 16 column chromatography.

3. The full length *lys 6-dh* gene from *A. denitrificans* was obtained by inverse PCR and cloned into *E. coli* BL21(DE3) using the expression vector, pET-17b. This gene has a single open reading frame of 1,107 bp nucleotides encoding the polypeptide of 368 amino acid residues.

4. The recombinant clones exhibited various levels of the specific activity from 1.33-1.93 units/mg protein. Moreover, the highest specific activity was 64 fold higher than that of *A. denitrificans* K-1. The induction with 0.2 mM IPTG for 4 hours showed the highest expression.

5. Despite of the recombinant clone (pET-ADK) was subcultured for 80 days, the *lys 6-dh* gene expression in *E. coli* BL 21(DE3) remained 100% activity of the parent. It implied that the recombinant clone had high stability of recombinant plasmid.

6. Lys 6-DH from pET-ADK clone was purified 2.8 fold with a 47% yield by procedure involving DEAE-Toyopearl and DEAE-Sephadex A50 column chromatography.

7. The molecular weight of the native Lys 6-DH was 80 kDa. In the presence of L-lysine, the molecular weight of Lys 6-DH was 240 kDa that consisted of 6 identical subunits (40 kDa).

8. The enzyme had high substrate specificity only on L-lysine while L-lysine analogs, L-ornithine, 6-amino-*n*-caproic acid and L-norluecine, were reduced Lys 6-DH activity to 70, 86 and 60%, respectively.

9. The enzyme required $NAD^+$ as a natural coenzyme for oxidative deamination. $NADP^+$ was inert while the $NAD^+$ analog, Nicotinamide guanine dinucleotide, showed 1.3 times higher activity than $NAD^+$.

10. The optimum pH and temperature of the Lys 6-DH was 9.3 and 50°C, respectively.

11. The enzyme was stable over a pH range from 7.5 to 8.0 in KPB (100% relative activity). In addition, the enzyme was fully stable at 30°C for 3 days and retained 50% of its activity after incubation for 9 days.

12. The apparent $K_m$ value for L-lysine and $NAD^+$ of dimeric and hexameric form of Lys 6-DH were 11.11, 0.138, 8.62 and 0.092 mM, respectively.

13. The full length of *p5cr* gene from *Bacillus cereus* ATCC 11778 was amplified and cloned into *E. coli* BL 21(DE3) using the expression vector, pET-17b. This gene has a single open reading frame of 782 bp nucleotides encoding the polypeptide of 260 amino acid residues.

14. The recombinant clones exhibited various levels of the specific activity from 1.24-4.75 units/mg protein. Moreover, the highest specific activity was 95 fold higher than that of *B. cereus*. The induction with 0.2 mM IPTG for 8 hours showed the highest expression.

15. The heterologous gene recombinant plasmid of *lys 6-dh* and *p5cr* genes were constructed using the expression vector, pET-17b and cloned into *E. coli* BL 21(DE3). The recombinant clones exhibited various levels of the specific activity from 0.27-0.75 and 0.10-0.54 units/mg protein of Lys 6-DH and P5CR activity, respectively. Moreover, the highest specific activity was 25 and 11 folds higher than that of their wild types. The induction with 0.1 mM IPTG for 3 hours showed the highest expression of both genes.

16. The amount of L-pipecolic acid was highest after 24 hours production using xylene treated recombinant cells for 5 min and using Tris-HCl, pH 9.0 as buffer. The maximum of L-pipecolic acid production was 77 mM (9.9 g/L) with 32.5% yield.