CHAPTER VI

CONCLUSIONS

Three strains of 4-chloroaniline-degradating bacteria, 4CA-2, 4CA-16, and 4CA-17, were isolated from tangerine groove and identified by using biochemical characterization based on methods in Manual of Clinical Microbiology and by using 16s rDNA sequence alignment. The results showed their species were *Acinetobacter baumannii, Pseudomonas putida, and Klebsiella pneumoniae*, respectively.

Acinetobacter baumannii (4CA-2) degraded 25 ppm (0.2 mM) 4-chloroaniline and showed percentage of total degradation of 61.00 ± 1.68% with a specific degradation rate of 8.70 ±1.60 nmol(min.mg protein)$^{-1}$. It could degrade 4-chloroaniline *via* a modified *ortho*-cleavage pathway which chlorocatechol 1,2-dioxygenase was expressed as 191.8 ± 47.5 nmol(min.mg protein)$^{-1}$. Catechol 1,2 dioxygenase activity was also detected with a specific activity 50.6 ± 17.5 nmol(min.mg protein)$^{-1}$ when it cultured on mineral medium containing 25 ppm (0.2 mM) 4-chloroaniline. Moreover, the additional carbon or/and nitrogen source which increased 4-chloroaniline degradation by 3.06%, 7.21% and 9.16% were 4 mM citrate, 4 mM NH_4Cl and the combination of both, respectively.

Pseudomonas putida (4CA-16) degraded 25 ppm (0.2 mM) 4-chloroaniline and showed percentage of total degradation of 59.82 ± 0.71 with a specific degradation rate of 13.60 ± 2.50 nmol(min.mg protein)$^{-1}$It could degrade 4-chloroaniline *via* a modified *ortho*-cleavage pathway which chlorocatechol 1,2-dioxygenase activity was expressed as 205.8 ± 68.6 nmol(min.mg protein)$^{-1}$.

Moreover, the additional carbon source which increased 4-chloroaniline degradation by 8.42% and 8.49% were 4 mM citrate and4 mM succinate.

Klebsiella pneumoniae (4CA-17) degraded 25 ppm (0.2 mM) 4-chloroaniline and showed percentage of total degradation of 62.82 ± 3.87 with a specific degradation rate 19.00 ± 2.30 nmol(min.mg protein)$^{-1}$. It could degrade 4-chloroaniline *via* a modified *ortho*-cleavage pathway which chlorocatechol 1,2-dioxygenase activity was expressed as 223.4 ± 35.7 nmol(min.mg protein)$^{-1}$. Moreover, the additional carbon or/and nitrogen source which increased 4-chloroaniline degradation by 5.56% and 4.37% were 1 mM and 2 mM aniline. The maximum percentage of total degradation to 62.82% and the specific degradation rate to 19.00 nmol (min.mg protein)$^{-1}$ was observed in *Klebsiella pneumoniae* (4CA-17).

For the substrate range, the isolates could degrade aniline, 2-chloroaniline, 3-chloroaniline to different extent but could not degrade 3,4-dichloroaniline.

The bacterial isolates were able to survive under the growth conditions used in the presence of 4-chloroaniline up to 1.2 mM. The growth and degradation were completely inhibited at 200 ppm (1.6 mM) of 4-chloroaniline.