論　文　の　要　旨

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論 文 題 目　Studies on a novel degradation pathway of 4-chloroaniline and chemotaxis to 4-chloroaniline in *Pseudomonas* strains
（Pseudomonas 細菌における新規 4-クロロアニリン分解経路と 4-クロロアニリン走化性に関する研究）

Chapter 1. General introduction

Chloroanilines (CAs) have been extensively used in industrial chemical production. They are also one of the primary intermediates generated by microbial transformation of herbicides. Because of their extensive application, CAs have been accumulated in environments. Due to their toxicity and recalcitrant properties, they have been considered as one of important environmental pollutants. To dissimilate the environmentally-contaminated CAs, bioremediation has been considered as a treatment option for detoxification. Bacterial chemotaxis is the important behavioral adaptation of bacteria to mediate a balance between nutrition and toxic effects surrounding chemicals. Chemotaxis to pollutants brings bacterial cells to areas at higher concentrations of pollutants, if pollutant-degrading bacteria have the ability to chemotatically respond to the pollutants. Therefore, chemotaxis to pollutants is expected to enhance biodegradation rate by increasing pollutant bioavailability. Objective of this study is to find and characterize a novel CA degradation pathway and to identify a chemotaxis sensory protein CAs.

Chapter 2. Degradation of 4-chloroaniline by toluene dioxygenase from *Pseudomonas putida* T57

I investigated the ability of *P. putida* toluene dioxygenase to oxidize CAs. Toluene-induced *P.putida* T57 cells degraded 4-chloroaniline (4CA) more rapidly than non-induced cells, suggesting that toluene dioxygenase pathway is involved in 4CA degradation. The recombinant *Escherichia coli* harboring *P. putida* T57 toluene dioxygenase gene (*todC1C2BA*) showed 4CA degradation activity, confirming involvement of toluene dioxygenase in 4CA degradation. Thin layer chromatography and mass spectrometry identified 4-chlorocatechol and 2-amino-5-chlorophenol as reaction products, suggesting that toluene dioxygenase catalyzes both 1,2 and 2,3-dioxygenation of 4CA. Then, I introduced plasmid containing the entire *tod* peron to *P. putida* T57 to enhance its ability to degrade 4CA. The resulting recombinant strain showed 250-fold higher 4CA degradation activity than the parental strain and completely degraded 2 mM 4CA within 2 hours. The recombinant strain also degraded 2-chloroaniline, 3-chloroaniline, and 3,4-dichloroaniline as well as 4CA, but

not 3,5-dichloroaniline. This is the first finding of 4CA degradation by toluene dioxygenase.

Chapter 3. Identification of CtpL as a chromosomally-encoded chemoreceptor for 4CA and catechol in *Pseudomonas aeruginosa* PAO1

I tested some *Pseudomonas* strains for their ability to chemotactically respond to 4CA and found that *P. aeruginosa* PAO1 showed significant attractive response to 4CA although it cannot utilize 4CA as a growth substrate. Molecular analysis of 26 mutants with a disrupted methyl-accepting chemotaxis protein gene revealed that CtpL, a chromosomally-encoded chemoreceptor, was responsible for the positive chemotatic responses to 4CA. Since CtpL has previously been described as a major chemoreceptor for inorganic phosphate at low concentrations, this is the first report of a fortuitous capability of CtpL to function towards aromatic pollutants. In addition, its regulation was not only dependent on the presence of the chemoattractant inducer, but was regulated by conditions of phosphate starvation.

Chapter 4. General conclusion

In summary, I have demonstrated that toluene dioxygenase in *P. putida* T57 oxidizes 4CA via oxidative deamination. This enzyme also oxidizes 2- and 3-CA, and 3,4-dichloroaniline. The ability of *P. putida* T57 to degrade 4CA could be greatly improved by introduction of additional *todC1C2BADE* genes. Then, I have found chemotaxis to 4CA and catechol in *P. aeruginosa* PAO1 and identified CtpL as a chemosensory protein for these attractants. These finding are useful to construct an efficient agent for bioremediation of CA-polluted environments.