B049

Analysis of Aromatic Catabolic Pathways in *Pseudomonas putida* KT 2440 by Combined Proteomic Approach: 2-DE/MS and ICAT Analysis

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The proteomic analysis of Pseudomonas putida KT2440 cultured in monocyclic aromatic compounds was performed to confirm the predicted aromatic compounds degradation pathways using the 2-DE/MS and ICAT analysis More than 100 protein spots were identified by 2-DE/MS or MS/MS analysis from cell cultured in five different culture conditions Benzoate dioxygenase(BenA, BenD) & catechol 1,2-dioxygenase(CatA) were induced by benzoate Protocatechuate 3,4-dixoygenase(PcaGH) were induced by p-hydroxybenzoate Same -ketoadipyl CoA thiolase(PcaF) and 3-oxoadipate CoA transferase(PcaI) were induced by benzoate, p-hydroxybenzoate and vanillne, suggesting that they were degraded by different dioxygenases and converged in the same –ketoadipate pathway. From comparative analysis of benzoate and succinate-induced proteome using ICAT methods, 110 proteins including seven benzoate-degrading enzymes were identified and complemented the 2-DE results Phenylethylamine induced -ketoacyl CoA thiolase(PhaD) and ring-opening enzyme(PhaL), enzymes of phenylacetate(pha) biodegradation pathway Alkyl hydroperoxide reductase(AphC) was induced in all aromatic compounds condition

B050

Cloning of *p*-Hydroxybenzoate Degradation Gene from *Pseudomonas* sp. K82

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Pseudomonas sp K82 cultured in *p*-hydroxybenzoate induced protocatechuate 4,5-dioxygenase (PCD 4,5) for the *p*-hydroxybenzoate degradation PCD4,5 was known to be heterodimer and high sequence homology with N-terminal sequences of PmdA and PmdB of *Comamonas testosteroni* In this study, 60 kbp EcoR1 fragments contained p-hydroxybenzoate degradation genes were cloned and sequenced Four genes were involved in the p-hydroxybenzoate biodegradation and three ORFs were additionally identified PCD4,5 was over-expressed and purified Our results suggest that gene structure and sequences of p-hydroxybenzoate degradation genes were homologous with those of Comamonas testosteroni

B051

Novel Catechol Genes for Benzoate Degradation from *Acinetobacter lwoffii* K24

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Acinetobacter lwoffii K24 known as an aniline degrading bacterium has been found to utilize other aromatic compounds such as p-hydroxybenzoate, salicylate and benzoate The proteome of *A. lwoffii* K24 under the growth of aniline had been analyzed in the previous study(Kim et al 2003), showing that two catechol 1,2-dioxygenases(U77658 & U77659) were induce for the aniline degradation In this study, we performed comparative 2-DE analysis of benzoate-induced proteome *A lwoffii* K24 induced new catechol 1,2-dioxygenase($CatA_3$) suggesting that new catechol 1,2-dioxygenase functions main dioxygenase for the benzoate degradation New cat genes have highest sequence homology with cat genes of A radioresistens and the gene structure of catBCAF This study provides evidence of multiple degradation pathways and regulation for the aromatic compound degradation in *A lwoffii* K24

B052

Tetracycline Resistance Proteome Analysis of *Pseudomonas putida* KT2440

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Tetracycline-induced proteome was analyzed from *Pseudomonas putida* KT2440 by 2-DE and MADLI-TOF-MS *P putida* KT2440 induced more than 30 protein spots under the condition of succinate media containing 50 g/ml tetracycline From the buffer-soluble fraction, proteins related with protein synthesis, peptidoglycan synthesis, gluconeogenesis, stress proteins and several ABC transporters were strongly induced From the buffer-insoluble fraction, enzymes for the synthesis of amino acid, fat acid and cofactor were identified Several proteins were common inducers of chemical stress These results suggest that during the survival of *P putida* KT2440 at high tetracycline condition, bacteria produced various metabolic, regulatory proteins and transporter as the stimulus response