S4-8

ANALYSIS OF GENE ORGANIZATION OF *PHN* OPERONS RESPONSIBLE FOR BIODEGRADATION OF POLYAROMATIC HYDROCARBONS (PAHs) BY *SPHINGOMONAS* SP. DJ77

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Sphingomonas sp. strain DJ77 can grow on phenanthrene, anthracene, naphthalene, biphenyl, toluene or *m*-xylene as the sole carbon source. We cloned and sequenced a 25 kb DNA fragment that encodes more than 23 PAHs-degrading enzymes. These structural genes are organized into four operons. The operon I contains eleven closely spaced genes (*phnDEGHIJKLMNO*) encoding enzymes mainly related to lower pathway degradation such as catechol 2,3-dioxygenase. The operon II contains five genes encoding two sets of iron sulfur proteins of dioxygenase subunits (*phnXYAB*) and glutathione S-transferase (*phnC*). The operon III contains more than five genes (*phnQRSTU*) encoding enzymes mainly related to upper pathway degradation such as 2,3-dihydroxybiphenyl-1,2-dioxygenase. The operon IV encodes xylene monooxygenase electron transfer subunit (*phnP*1) and xylene monooxygenase hydroxylase subunit (*phnP*2).

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SUBSTRATE-DEPENDENT EXPRESSION OF *ORTHO*- AND *META*-CLEAVAGE PATHWAYS IN *SPHINGOMONAS YANOIKUYAE* B1

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Catabolic pathways for the degradation of various aromatics by *Sphingomonas yanoikuyae* B1 are interwined, joining at the level of (methyl)benzoate and catechol, which is further degraded via a ring cleavage reaction. A deletional mutant strain *S. yanoikuyae* EK497, was constructed by deleting approximately 35 kb genomic DNA region containing the degradative genes including one for a putative transcriptional regulator (*bphR*). EK497 is unable to grow on all of the aromatics tested except for benzoate. When grown on benzoate EK497 possesses a significant amount of *ortho*-cleavage activity without any *meta*-cleavage activity, but B1 has both activities. This means that both *ortho*- and *meta*-cleavage pathways for benzoate degradation are present in B1. However, *m*-toluate fails to induce the *ortho*-cleavage pathway suggesting the presence of a substrate-dependent induction mechanism. In order to investigate the putative regulatory mechanism in more detail at the molecular level, insertional mutant strain *S. yanoikuyae* JS81 (*bphR*::Km) was constructed. Interestingly, the magnitude of *ortho*-cleavage activity in JS81 is approximately tenfold lower than that in B1 when grown on benzoate. These results indicate that BphR acts as a positive regulator for the expression of the *ortho*-cleavage pathway in B1.