E327 Presence of an Alternative Metabolic Pathway for Benzoate Degradation in the PAH-degrading *Sphingomonas yanoikuyae* B1

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Sphingomonas yanoikuyae B1 is versatile in its ability to metabolize a wide spectrum of aromatic hydrocarbons as the sole carbon and energy sources such as benzoate, *m*-toluate, *m*-xylene, toluene, biphenyl, naphthalene, and phenanthrene. The catabolic pathways for the degradation of both the monocyclic and polycyclic aromatic hydrocarbons (PAHs) are interwined, joining at the level of (methyl)benzoate and catechol, which is further degraded via a TOL plasmid type *meta*-cleavage pathway. A deletional mutant strain, *S. yanoikuyae* EK497, was constructed by deleting approximately 35 kb genomic DNA region containing the degradative genes. EK497 is unable to grow on the above aromatics tested except for benzoate. B1 metabolizes benzoate with development of bright yellow color, which is indicative of *meta*-ring cleavage. In contrast, EK497 grows even faster without the color development. Both *meta*- and *ortho*-cleavage catechol dioxygenases activities were examined in crude cell extracts of B1 and EK497 grown on benzoate. EK497 possesses a significant amount of *ortho*-cleavage activity, but no *meta*-cleavage activity. This means that an alternative *ortho*-cleavage pathway for benzoate degradation is present in EK497, which may not be operated efficiently in the presence of the *meta*-cleavege pathway.

E328 Screening of small molecular β-lactamase inhibitor from *Actinomycetes* groups.

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The aim of this study is screening of microorganisms which produce small molecular β-lactamase inhibitor. Since the lysine ε-aminotransferase (LAT) is the key enzyme for the biosynthesis of β-lactam compounds, the gene (*lat*) encoding lysine ε-aminotransferase was chosen as a reporter gene for the molecular screening of microorganisms. The internal fragment of the *lat* gene labeled with horseradish peroxidase was used as a probe.

Agar diffusion bioassay was performed to confirm β-lactamase inhibitory activity. Ultrafiltration kit was used to select the microorganism which produces small molecular β-lactamase inhibitor.

The inhibitory effects of the culture supernatant of soil isolates on the activity of β-lactamase were quantitated using synthetic substrate, PADAC (Pyridine-2-Azo- p-Dimethylaniline Cephalosporine). Finally, one strain was chosen as a candidate for producing small molecular β-lactamase inhibitor.