

Expression of Novel Salicylate Oxygenase Genes from a *Sphingomonas* strain in *E.coli*

Ok-young Cho¹, Si Wouk Kim², and Eungbin Kim¹

¹Department of Biology and Institute of Life Science and Biotechnology,
Yonsei University, Seoul 120-749, Korea

²Department of Environmental Engineering, Chosun University, Kwangju, 501-759, Korea

Bacterial aromatic-ring dioxygenase is a normally three-component enzyme system, which consists of a flavoprotein reductase, a ferredoxin and an iron sulfur protein (terminal oxygenase).

Sphingomonas yanoikuyae B1 is unique in that it possesses least six different sets of an iron sulfur protein component, which are apparently associated with a single ferredoxin (BphA3) and a reductase (BphA4) components. Previous studies suggested that the gene for one of the multiple oxygenases (*bphA1cA2c*) is responsible for metabolizing salicylate and 1-hydroxy-2-naphthoate during the degradation of naphthalene and phenanthrene (Kim et al., Abstr. Am. Soc. Microbiol., Q299, p. 470. 1998). To investigate the function of the *bphA1cA2c* gene in more detail, the genes for *bphA1cA2c* and *bphA3* were amplified by PCR, and cloned together into an expression vector. The *Escherichia coli* strain harboring the recombinant plasmid converted salicylate, 3-methylsalicylate, 4-methylsalicylate, or 5-methylsalicylate to catechol, 3-methylcatechol, 4-methylcatechol, or 4-methylcatechol, respectively as confirmed by UV-visible spectral, HPLC, and GC/mass-spectrometric analyses. Furthermore, this expression system was also able to oxidize benzoate, *m*-toluate, or 1-hydroxy-2-naphthoate although the metabolite structures have not been identified yet.