

E317 **Characteristics of Catechol 2,3-Dioxygenase produced by 4-chlorobenzoate degrading *Pseudomonas* sp. S-47**

Ki-Pil Kim^{*} and Chi-Kyung Kim
Department of Microbiology, Chungbuk National University

Pseudomonas sp. S-47 is capable of transforming 4-chlorobenzoate to form 4-chlorocatechol which is subsequently oxidized by *meta*-cleavage dioxygenase to produce 5-chloro-2-hydroxymuconic semialdehyde. Catechol 2,3-dioxygenase catalyzing *meta*-cleavage of 4-chlorocatechol was produced from the organism, purified, and characterized. The C23O enzyme was maximally produced in the late logarithmic growth phase, and the maximum temperature and pH for the enzyme activity were 30~35°C and 7.0, respectively. The enzyme could be purified and concentrated by 5 fold from the crude cell extract through the steps of the Q sepharose chromatography and Sephadex G-100 gel filtration after acetone precipitation. The enzyme was examined to be consisted of 35 kDa subunits when analyzed by SDS-PAGE. The C23O produced by *Pseudomonas* sp. S-47 was similar to XylE of *Pseudomonas Putida* in substrate specificity for several catecholic compounds.

E318 **Degradation of 4-Chlorobenzoate by *Pseudomonas* sp. S-47 Isolated from Contaminated Waste**

Dong-In Seo, Jong-Chan Chae, Ki-Pil Kim^{*} and Chi-Kyung Kim
Department of Microbiology, Chungbuk National University

A bacterial strain degrading 4-chlorobenzoate (4CBA) under aerobic condition was isolated. The strain was identified as *Pseudomonas* species by morphological and biochemical studies. The growing cell assay with *Pseudomonas* sp. S-47 showed that 4-chlorobenzoate was degraded to form yellow-colored *meta*-cleavage product. The product was proved to be 5-chloro-2-hydroxymuconic semialdehyde (5C-2HMS) by GC-mass spectrometry as well as UV-visible spectrophotometry, which was produced by dioxygenation of 4-chlorocatechol (4CC). Dechlorination of 5-chloro-2-hydroxymuconic acid (5C-2HMA) by *Pseudomonas* sp. S-47 was accompanied by simultaneous formation of 2-hydroxypenta-2,4-dienoic acid (2HP-2,4DA). This indicates that *Pseudomonas* sp. S-47 degrades 4-chlorobenzoate to 2HP-2,4DA via a novel catabolic pathway of 4CC conducting by sequential reactions of *meta*-cleavage and dechlorination.