

Aerobic Degradation of Tetrachloroethylene (PCE)
by *Pseudomonas stutzeri* OX1

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ABSTRACT

Since trichloroethylene (TCE), dichloroethylene (DCE), and vinyl chloride (VC) arise from anaerobic degradation of tetrachloroethylene (PCE) and TCE, there is interest in creating aerobic remediation systems that avoid the highly toxic VC and *cis*-DCE which predominate in anaerobic degradation. However, it seemed TCE could not be degraded aerobically without an inducing compound (which also competitively inhibits TCE degradation). It has been shown that TCE induces expression of both the toluene dioxygenase of *P. putida* F1 as well as toluene-*p*-monooxygenase of *P. mendocina* KR1. We investigated here the ability of PCE, TCE, and chlorinated phenols to induce toluene-*o*-xylene monooxygenase (ToMO) from *P. stutzeri* OX1. ToMO has a relaxed regio-specificity since it hydroxylates toluene in the *ortho*, *meta*, and *para* positions; it also has a broad substrate range as it oxidizes *o*-xylene, *m*-xylene, *p*-xylene, toluene, benzene, ethylbenzene, styrene, and naphthalene; chlorinated compounds including TCE, 1,1-DCE, *cis*-DCE, *trans*-DCE, VC, and chloroform; as well as *mixtures* of chlorinated aliphatics (*Pseudomonas* 1999 Maui Meeting). ToMO is a multicomponent enzyme with greatest similarity to the aromatic monooxygenases of *Burkholderia pickettii* PKO1 and *P. mendocina* KR1. Using *P. stutzeri* OX1, it was found that PCE induces

P. mendocina KR1. Using *P. stutzeri* OX1, it was found that PCE induces ToMO activity measured as naphthalene oxygenase activity 2.5-fold, TCE induces 2.3-fold, and toluene induces 3.0 fold. With the mutant *P. stutzeri* M1 which does not express ToMO, it was also found there was no naphthalene oxygenase activity induced by PCE and TCE; hence, PCE and TCE induce the *tou* path. Using *P. putida* PaW340(pPP4062, pFP3028) which has the *tou* promoter fused to the reporter catechol-2,3-dioxygenase and the regulator gene *touR*, it was determined that the *tou* promoter was induced 5.7-, 7.1-, and 5.2-fold for 2-, 3-, 4-chlorophenol, respectively (cf. 8.9-fold induction with *o*-cresol); however, TCE and PCE did not directly induce the *tou* path. Gas chromatography and chloride ion analysis also showed that TCE induced ToMO expression in *P. stutzeri* OX1 and was degraded and mineralized. This is the first report of significant PCE induction of any enzyme as well as the first report of chlorinated compound induction of the *tou* operon. The results indicate TCE and chlorinated phenols can be degraded by *P. stutzeri* OX1 without a separate inducer of the *tou* pathway and without competitive inhibition.
