AEROBIC BIOTRANSFORMATION OF PENTACHLOROPHENOL BY *PSEUDOMONAS CHLORORAPHIS*

In-Hyun Nam, Hyo-Bong Hong, Sung-Deuk Choi, Yoon-Seok Chang

School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang, Korea.

Introduction

Pentachlorophenol (PCP) is a priority pollutant that is widely used as a general biocide in commercial wood treatment. Pentachlorophenol is toxic and, in some cases, highly persistent priority pollutant in the environment. This compound has been used on a large scale serving as wood preservatives, pesticides, and precursors of herbicides.[1,2] PCP would be expected to be recalcitrant to biodegradation for two reasons. First, it is highly chlorinated. Usually, the resistance of aromatic xenobiotics to biodegradation generally increases with the number of chlorine substituents. Second, it is very toxic because it uncouples oxidative phosphorylation and perturbs membrane properties[3]. However, various microorganisms have been isolated and their metabolic pathways have also been elucidated[4,5]. Our study showed that PCP transformation begins with initial dechlorination and hydroxylation reactions which form tetrachlorophenol and tetrachlorocatechol as metabolic intermediates. The novelty of the catabolic ability of *P. chlororaphis* and its potential importance for degradation of highly chlorinated phenols in the environment led us to investigate its ability to degrade pentachlorophenol at high concentrations.

In this study, we isolated a bacterium, *Pseudomonas chlororaphis*, which was able to produce tetrachlorophenol and tetrachlorocatechol by the degradation of pentachlorophenol (PCP) and identified their metabolic intermediates using gas chromatography-ion trap-massspectrometry (GC-IT-MS). Furthermore, the quantitative survey of pentachlorophenol was performed by GC-ECD (gas chromatograph equipped with an electron capture detector) to measure the decrease of substrate. Consequently our results showed an inhibitory effect of tetrachlorocatechol, a compound of metabolic intermediates. The further study with oxygen uptake rates determined.

Methods and Materials

Degradation Experiments. We isolated a bacterium strain, *Pseudomonas chlororaphis*, capable of converting PCP to tetrachlorophenol (TeCP) and tetrachlorocatechol (TeCC). The mineral salts medium had the following composition (in grams per liter of distilled water) : Na_2HPO_4-$2H_2O$ 2.2 g, KH_2PO_4 0.8 g, NH_4NO_3 3.0 g, $Fe_2(SO_4)_3$-H_2O 0.01 g, $CaCl_2$-$2H_2O$ 0.01 g, $MgCl_2$ 0.01 g. *P. chlororaphis* was grown on the mineral salts medium with yeast extracts at concentration of 0.05 g/L for 16 hours (30°C, 150 rpm). After incubation, cells were harvested by centrifugation at 8,000 rpm for 20 minutes, washed three times with 20 mM phosphate buffer (pH 7.0). Then *P. chlororaphis* strain was inoculated on the mineral salts medium containing pentachlorophenol at concentration of 0.3 mM as a sole source of carbon and energy. Uninoculated and heat killed culture served as parallel controls. Actually, inoculated strain turbidity was determined about 0.02 in a pentachlorophenol mixed mineral salts medium. Temperature, optical density, chloride concentration, and pH were measured periodically.

Sample extraction and analysis. The supernatants of culture solution were extracted with an equal volume (100 ml) of ethyl acetate, followed by centrifugation (3,500 rpm for 20 minutes) and filtration using 0.45 μm pore size filters. An acidified sample was obtained by adding 85% *ortho*-phosphoric acid to the sample until the pH was adjusted to 2.0. Extracted samples were dried over anhydrous sodium sulfate and evaporated by gently blowing N_2 gas. Gas chromatography-mass spectrometry (GC/MS) was carried out on a Finnigan GC-IT(ion trap)-MS. Analytical conditions of GC-IT-MS for the separation and fragmentation were previous described[6].

The quantification of reduced pentachlorophenol during degradation experiments was performed by HP 6890 gas chromatograph equipped with electron capture detector(ECD) and DB-5 (30 m) capillary column. Metabolic intermediates were extracted from the supernatants of culture with a 50 ml of ethyl acetate, followed by centrifugation (3,500 rpm for 20 minutes) and filtration using 0.45 μm pore size filters. Then, all samples were extracted for 10 hours in shaking incubator at 170 rpm. After shaking, organic phases were taken from all samples. To dry samples, anhydrous sodium sulfate, previously dried at 450°C furnace for overnight, was added.

Results and Discussion

In order to confirm whether the isolated strain can utilize PCP as a sole source of carbon and energy, PCP-grown cells of *P. chlororaphis* were washed and cultured in MSM containing 0.3 mM PCP. As shown in Fig. 1, PCP levels decreased with culture time and cell density gradually increased. PCP levels were reduced to an amount corresponding to a total substrate concentration of 0.2 mM, with almost linear responses to the culture turbidity, chloride release, and decrease in pH. Further degradation of PCP did not occur because of the inhibitory effects of metabolic intermediates. We investigated the metabolic intermediates by degradation of pentachlorophenol (PCP) during the incubation period. The mass spectra of the metabolic intermediates are shown in Figs. 2 and 3. These mass spectra contain peaks that suggest that the degradation products of pentachlorophenol are tetrachlorophenol (TeCP) and tetrachlorocatechol (TeCC). The formation of TeCP was confirmed through this experiment from the 12 hours later. This suggests that the major initial metabolic intermediate is TeCP, formed by a reductive dechlorination reaction. However, the formation of TeCC was observed 72 hour later during incubation periods. This suggests that the second metabolic intermediate is TeCC formed by a hydroxylation reaction. TeCC was not transformed, which caused the degradation process to decelerate and no more metabolic intermediates to be produced. In addition, the second metabolite, tetrachlorocatechol, was produced small amount from final period of incubation, transfer its inhibitory effects in the medium. This was further confirmed by GC-MS analysis, in which samples were incubated with TeCC standards and analyzed for various TeCC isomers.

Acknowledgements

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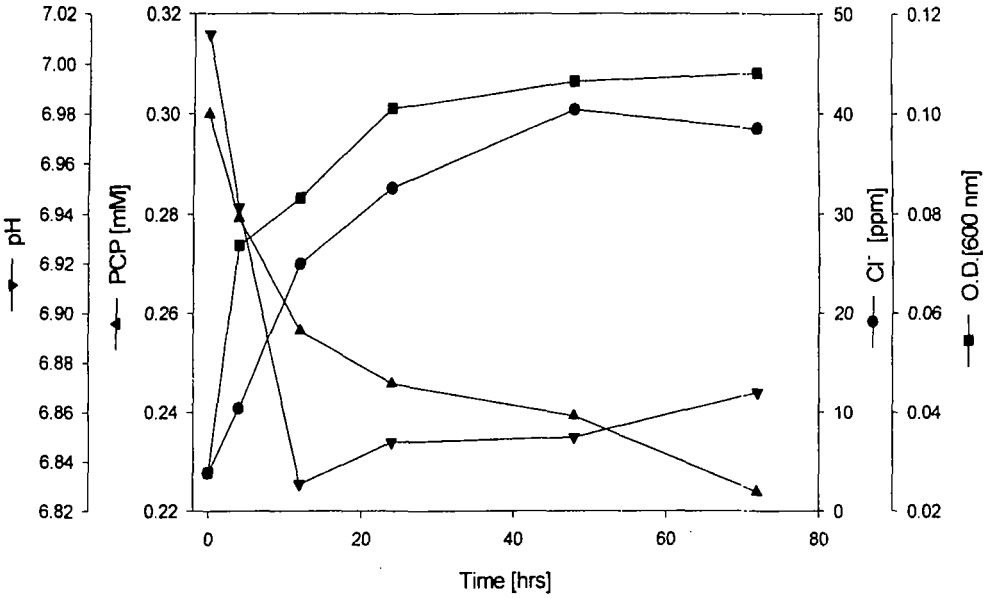

Fig. 1. Growth of *P. chlororaphis* with PCP. The initial concentration of dissolved carbon source (PCP) in the culture fluid was 0.3 mM.

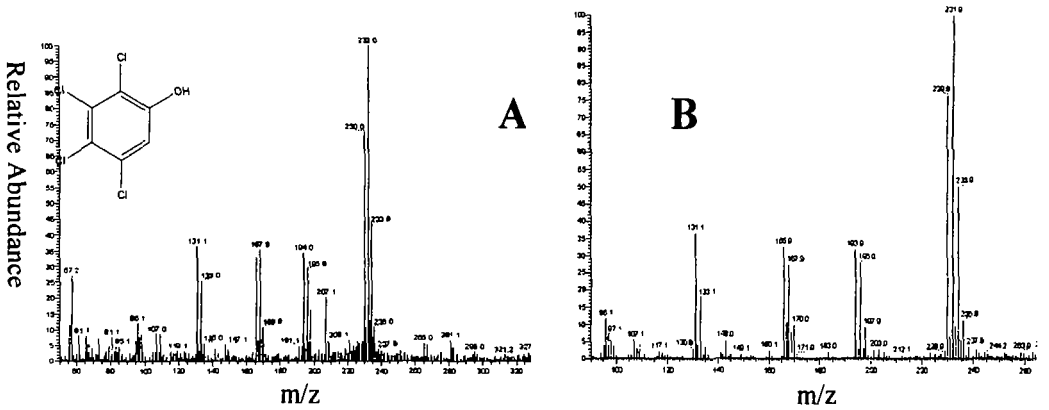

Fig. 2. Mass spectra of authentic TeCP (A) and metabolite (B).

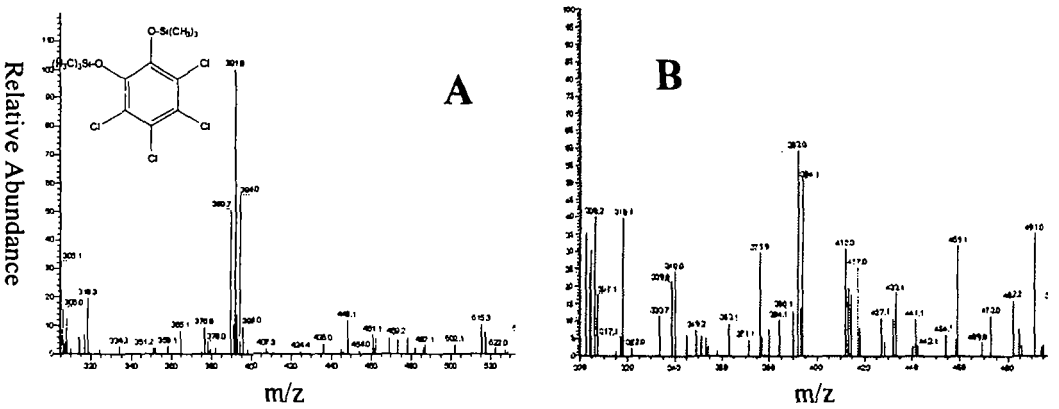

Fig. 3. Mass spectra of TMS-derivatized authentic TeCC (A) and TMS-derivatized metabolite (B).