Biodegradation **7**: 463–469, 1996.

Biodegradation of the mixtures of 4-chlorophenol and phenol by *Comamonas testosteroni* CPW301

Hee Sung Bae[1], James M. Lee[2], Young Bae Kim[3] & Sung-Taik Lee[1]*
[1] *Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejun, Korea;*
[2] *Department of Chemical Engineering, Washington State University, Pullman, WA, USA;* [3] *Department of Food Technology, Korea University, Seoul, Korea (* corresponding author, email: stlee@sorak.kaist.ac.kr)*

Accepted 17 December 1996

Key words: 4-chlorophenol and phenol mixture, degradation, *meta*-cleavage pathway

Abstract

A 4-chlorophenol (4-CP)-degrading bacterium, strain CPW301, was isolated from soil and identified as *Comamonas testosteroni*. This strain dechlorinated and degraded 4-CP via a *meta*-cleavage pathway. CPW301 could also utilize phenol as a carbon and energy source without the accumulation of any metabolites via the same *meta*-cleavage pathway. When phenol was added as an additional substrate, CPW301 could degrade 4-CP and phenol simultaneously. The addition of phenol greatly accelerated the degradation of 4-CP due to the increased cell mass. The simultaneous degradation of the 4-CP and phenol is useful not only for enhanced cell growth but also for the bioremediation of both compounds, which are normally present in hazardous waste sites as a mixture.

Introduction

Polychlorinated phenols have been released into the environment because of their wide usage as biocides, wood preservatives, and organic precursors in the synthesis of chlorophenoxyacetate herbicides. They are frequently transformed by anaerobic microorganisms to various lower chlorinated phenols, which can be further dechloriated to 4-chlorophenol (4-CP) or phenol (Boyd & Shelton 1984; Cole et al. 1994; Mikesell & Boyd 1986). Since 4-CP cannot be easily degraded in anaerobic conditions (Madsen & Aamand 1992; Woods et al. 1989), 4-CP and phenol may constitute two major byproducts of the anaerobic polychlorophenol degradation. Therefore, it is useful for a microorganism to degrade both compounds simultaneously.

It has been reported that 4-CP can be totally mineralized by aerobic microorganisms such as *Pseudomonas* sp. B13 and *Alcaligenes* sp. A7-2 through an *ortho*-cleavage pathway (Balfanz & Rehm 1991; Knackmuss & Hellwig 1978; Westmeier & Rehm 1987). On the other hand, phenol is known to be degraded completely via a *meta*-cleavage pathway (Feist & Hegeman 1969; Murray et al. 1972). Therefore, it is difficult for a single microorganism to degrade the mixture of the two compounds. Menke and Rehm (1992) reported that the mixture of 4-CP and phenol can be degraded by *Alcaligenes* sp. A7-2, however, phenol degradation was incomplete due to the accumulation of an intermediate metabolite, catechol.

In this study, we report the complete degradation of the 4-CP and phenol mixture by a newly isolated strain via a *meta*-cleavage pathway.

Materials and methods

Medium and chemicals

Minimal salt medium (MSM) consisted of the following components per liter: 1 g K_2HPO_4, 0.6 g NaH_2PO_4, 1 g NH_4NO_3, 0.2 g $MgSO_47H_2O$, 0.2 g KCl, 0.002% yeast extract, and 1 ml trace element solution (Lee et al. 1991). $MgSO_47H_2O$ and KCl were sterilized separately and then added to the cooled medium to prevent precipitation. The medium pH was adjusted to 7.3 by the addition of 1 N NaOH. The chlorophenols used in this study were obtained from Aldrich (Milwaukee, Wis-

consin). Methylcatechols and 4-chlorocatechol were purchased from Kasei (Tokyo, Japan) and Nacalai Tesque (Kyoto, Japan), respectively.

Isolation and characterization of a 4-CP degrading bacterium

A strain of bacterium capable of degrading 4-CP was isolated from soil samples collected from industrial stream sites. The soil samples were suspended in MSM (10 g soil/l) supplemented with 4-CP (50 mg/l) in 500-ml Erlenmeyer flasks on a shaker (120 rpm) at 30 °C, which was subcultured every 7 days until the growth was evidenced by the increase of turbidity. Single colony was isolated by spreading 0.1 ml of the enrichment culture on 4-CP agar plates.

The isolate was identified on the basis of its morphological, physiological, and chemotaxonomical properties. Gram staining, catalase, oxidase, oxidation-fermentation, and poly-β-hydroxybuthylate accumulation were tested according to the procedures outlined in 'Manual of Methods for General Bacteriology' (Smibert & Krieg 1981). DNA base composition was determined by reverse-phase HPLC (Waters Associates, Massachusetts) after enzymatic hydrolysis of the DNA into nucleosides (Tamaoka & Komagata 1984). Cellular morphology and flagella were observed using a transmission electron microscope after staining with 2% ammonium molybdate. The electron micrographs were taken by using a Philips C.M. 20 at 80 kV. API 20NE (BioMerieux SA, Lyon, France) and the Microbact System (Disposable Products Pty., Ltd., Adelaide, Australia) were used for other detailed characteristics.

Culture conditions

One or two colonies of cells grown on nutrient agar were inoculated in 50 ml of MSM in 500-ml Erlenmeyer flasks and shaken at 120 rpm and 30 °C for about 16 h (overnight culture). All batch experiments were initiated by adding suitable carbon sources to the overnight culture. For the washed cell experiments, cells were cultured in MSM containing 0.5 mM 4-CP or phenol and were washed twice with 50 mM phosphate buffer (pH 7.0). Washed cells were resuspended in the phosphate buffer with one of monochlorophenols (0.5 mM), and incubated at 120 rpm and 30 °C.

Analytical methods

Cell growth was determined by measuring light absorbency at 600 nm with a spectrophotometer (Beckman, Model DU-68, Fullerton, California). The absorbency was converted to dry weight by using a standard curve. The specific 4-CP degradation rate was calculated as the change of 4-CP concentration divided by time required and by average cell concentration, $\Delta S/(\Delta t\, X)$. Chloride ion concentration was determined by using a pH meter (Orion, no. 701A, Boston, Massachusetts) with a chloride specific electrode. The standard chloride concentration was calibrated with NaCl in minimal salts medium before measurement. The concentrations of chlorophenols and phenol in culture fluid were determined with a reverse-phase HPLC equipped with a Nova pack C_{18} column (3.9 × 150 mm) and a UV detector set at 280 nm. The mobile phase was composed of methanol-water-acetic acid (100:100:2, v:v:v).

Metabolite formation during 4-CP degradation was monitored by scanning a culture medium sample with spectrophotometer (Beckman, Model DU-68, Fullerton, California) in the UV range. When detectable levels of metabolite appeared, the supernatant of a culture sample was acidified (pH 2.0) with HCl and the metabolites were extracted with ethyl acetate. The extracted organic layer was dried over sodium sulfate beads and then evaporated to a small volume. The extracted metabolites were treated with *N*-methyl-*N*- trimethylsilyl trifluoroacetamide to generate trimethylsilyl derivatives and analyzed with gas chromatograph-mass spectrometry (Fisons, Altrincham, England). The ion energy in the mass spectrometry was 70 eV. The oven temperature was kept at 100 °C for 2 min and then increased to 280 °C at the rate of 20 °C per minute. A fused-silica capillary column was coated with SE-30-cross-linked methylsilicone (25 m × 0.25 mm I.D.).

Measurement of enzyme activities for ring fission

Cells were washed with 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM ascorbic acid and resuspended in the same buffer. The cells were broken by means of ultrasonic treatment in an ice bath and centrifuged at 20,000 × *g* for 30 min at 4 °C. The supernatant was used as a crude enzyme extract immediately afterward.

Catechol 2,3-dioxygenase activity was determined spectrophotometrically by measuring the formation of *meta* cleavage reaction products at 25 °C in 50 mM

phosphate buffer containing 100 μM of catechol or substituted catechols. Specific activities were calculated by using the following extinction coefficients ε for the *meta* cleavage of each reaction product: catechol, ε = 36,000 at 375 nm; 3-methylcatechol, ε = 32,000 at 388 nm; 4-methylcatechol, ε = 32,000 at 382 nm; 4-chlorocatechol, ε = 40,000 at 379 nm (Asturias & Timmis 1993).

Catechol 1,2-dioxygenase activity was determined spectrophotometrically by measuring the formation of *ortho* cleavage reaction products at 25 °C in 33 mM Tris/HCl (pH 8.0) containing 100 μM of catechol or substituted catechols, 1.3 mM of EDTA, and 3.3 mM of 2-mercaptoethanol. Specific activities were calculated by using the following extinction coefficients at 260 nm: catechol, ε = 16,800; 4-methylcatechol, ε = 13,900; 4-chlorocatechol, ε = 12,400 (Dorn & Knackmuss 1978).

One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of product per min in 1 ml reaction mixture. Specific activities were expressed as units per mg of protein. Protein concentration was measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

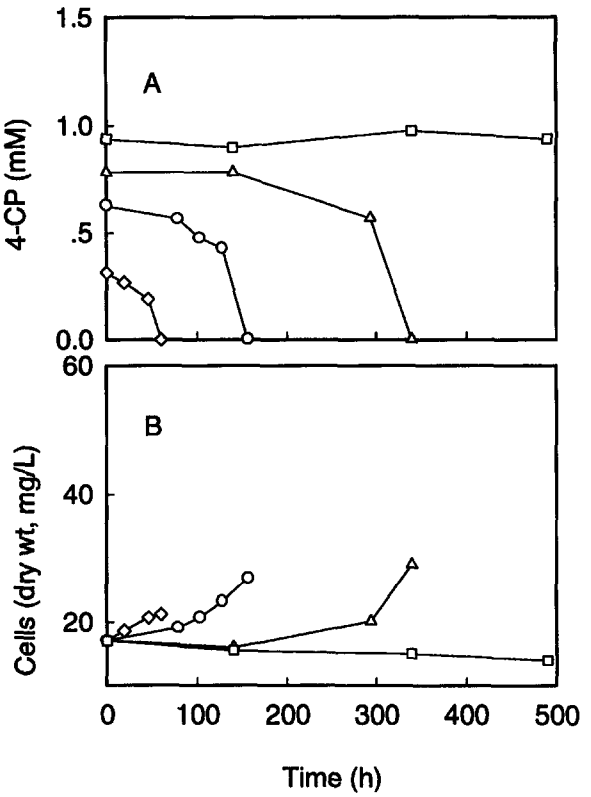

Figure 1. Effect of initial 4-CP concentrations on (*A*) 4-CP degradation and (*B*) cell growth in batch cultures.

Results

Isolation and characterization of a 4-CP-degrading bacterium

A 4-chlorophenol-degrading bacterium CPW301 was isolated from soil contaminated with industrial waste water in Choung-Ju, Korea. The phenotypic and chemotaxonomic characteristics of the isolate were consistent with *Comamonas testosteroni* (Tamaoka et al. 1987). CPW301 was able to cleave protocatechuate at the *meta*-position and grew well without organic growth factors. On solid nutrient agar, CPW301 formed colonies of 2–3 mm in diameter after incubation at 30 °C for two days. The strain was subcultured in a complex nutrient agar medium (Difco) without losing the 4-CP-degrading activity.

To test the degradability of other chlorophenol isomers, CPW301 was cultivated in MSM containing one kind of chlorophenol at a concentration of 10 mg/l. It was found that CPW301 could not degrade any other chlorophenols: 2-,3-chlorophenol; 2,4-2,5-2,6-3,4-3,5-dichlorophenol; 2,3,4-,2,3,5-,2,3,6-,2,4,5-,2,4,6-trichlorophenol; or pentachlorophenol. On the contrary, phenol (50 mg/l) was degraded completely by CPW301 without the accumulation of any metabolites.

Biodegradation of 4-CP

Figure 1 shows the 4-CP degradation and the growth of the strain CPW301 in MSM containing 4-CP at different concentrations. The cell concentration increased as 4-CP was degraded. The duration of the lag phase increased as the initial 4-CP concentration increased. When the initial 4-CP concentration increased to 0.9 mM, the lag phase increased to such an extent that the growth phase was not initiated within 500 h. The growth yield was about 0.11 (g dry cells/g 4-CP) when the initial 4-CP concentration was less than 0.9 mM. In all cultures, 4-CP was degraded completely, since the amount of chloride accumulated was equimolar to the initial amount of 4-CP in the medium (data not shown).

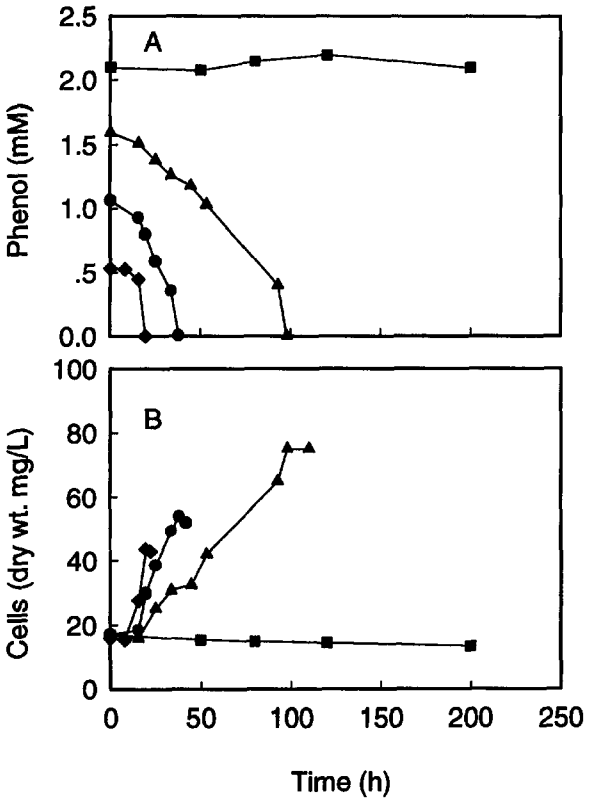

Figure 2. Effect of initial phenol concentrations on (*A*) phenol degradation and (*B*) cell growth in batch cultures.

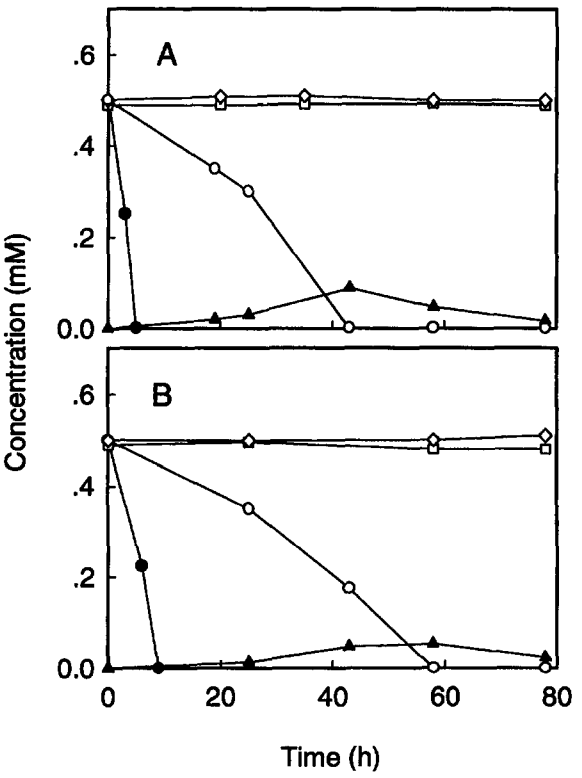

Figure 3. Degradation of phenol and monochlorophenols by (*A*) 4-CP-grown or (*B*) phenol-grown cells. The experiment was initiated by adding phenol or monochlorophenols to the washed cell suspension (0.51 g dry cells l^{-1}). Other conditions are the same as batch cultures. Symbols: □, 2-CP; ◇, 3-CP; ○, 4-CP; ●, phenol.

Intermediate metabolites during 4-CP degradation

During the 4-CP degradation, the culture medium turned a greenish-yellow color (maximum absorption at 378 nm), which indicated the production of intermediate compounds. The colored intermediate was identified to be 5-chloro-2-hydroxy muconic semialdehyde on the basis of its mass spectrum with the composition of the primary fragment ions at *m/z* 305 (M-CH_3), 285 (M-Cl), 231 (M-OTMS), 203 (M-COO-TMS), and 167 (M-OTMS-Cl-CHO). These molecular ions at *m/z* 305, 231, and 203 had the characteristic M/M +2 ratio of 3:1 resulting from the $^{35}Cl/^{37}Cl$ isotope ratio of a single Cl atom. This mass spectrum is consistent with that previously reported for the same compound (Sondossi et al. 1992).

The amount of the *meta*-cleavage product was less than about 12% of the initial 4-CP concentration, which was calculated from its molar extinction coefficient (ε = 40,000 at 379 nm). After the complete depletion of 4-CP, the colored intermediate slowly disappeared.

Degradation of phenol

Figure 2 shows the phenol degradation and the growth of the strain CPW301 in MSM containing phenol at different concentrations. As the initial phenol concentration increased, cell growth was not affected as much as in the case of 4-CP, though the cell growth stopped when the initial phenol concentration was 2.1 mM. The maximum substrate concentration for cell growth and phenol degradation was about 1.5 mM. The growth yield was calculated to be 0.42–0.58 (g dry cells/g phenol) in the tested range, which was about five times higher than the yield when 4-CP was used.

Washed cell experiments for the degradation of 4-CP and phenol

To see if cells induced with 4-CP could utilize phenol, we examined the disappearance of phenol by using the washed cells which were cultured in MSM containing

0.5 mM 4-CP or phenol. Additionally, the possibility of the disappearance of other monochlorophenols was also tested. Figure 3 shows the degradation of phenol and chlorophenols by phenol- or 4-CP-grown cells. Phenol-grown cells could degrade both 4-CP and phenol (Figure 3A), and the 4-CP grown cells did likewise (Figure 3B). When 4-CP was added as a substrate, the *meta*-cleavage product was temporarily accumulated in both types of cell suspension fluid.

The other monochlorophenolic compounds, 3-CP and 2-CP, could not be metabolized by the phenol- or 4-CP-grown cells. This indicated that the metabolic pathways for the degradation of 3-CP and 2-CP were different from those for phenol or 4-CP.

Ring cleavage enzymes

In order to find the initial pathway for the degradation of phenol and 4-CP by CPW301, two key enzymes catalyzing ring fission (catechol 2,3-dioxygenase and catechol 1,2-dioxygenase) were assayed by using catechols as substrates. Table 1 shows the specific activities of the two key enzymes extracted from different kinds of cells grown with 4-CP, phenol, or glucose as their main substrates. The cells grown on both phenol and 4-CP showed high catechol 2,3-dioxygenase activity, while they did not show any catechol 1,2-dioxygenase activity. After prolonged incubation, all *meta*-cleavage products were metabolized by the crude enzyme extracts. Cells grown with glucose as a main carbon source showed very low activities of both catechol 2,3-dioxygenase and catechol 1,2-dioxygenase.

Simultaneous degradation of 4-CP and phenol

To determine the strain's ability to degrade 4-CP and phenol simultaneously, CPW301 was incubated with an equimolar amount of 4-CP and phenol at three different initial concentrations (0.3, 0.6, and 0.98 mM). As shown in Figure 4, both 4-CP and phenol could be degraded simultaneously by the strain. The addition of phenol greatly accelerated 4-CP degradation. For example, when the initial concentration of 4-CP was 0.6 mM, the complete degradation took about 150 h for 4-CP alone (Figure 1) compared to 70 h for the 4-CP and phenol mixture (Figure 4).

Figure 5 shows the effect of different initial phenol concentrations when the initial 4-CP concentration was fixed. The addition of phenol shortened the time required for the degradation of 4-CP when Figure 5 was compared with Figure 1. The increased phenol concen-

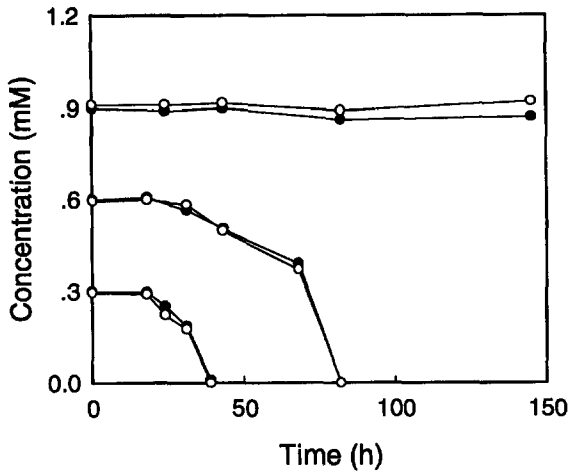

Figure 4. Degradation of 4-CP and phenol mixture in batch cultures. Initial cell concentration was 17 mg dry cells l^{-1}. Symbols: ○, 4-CP; ●, phenol.

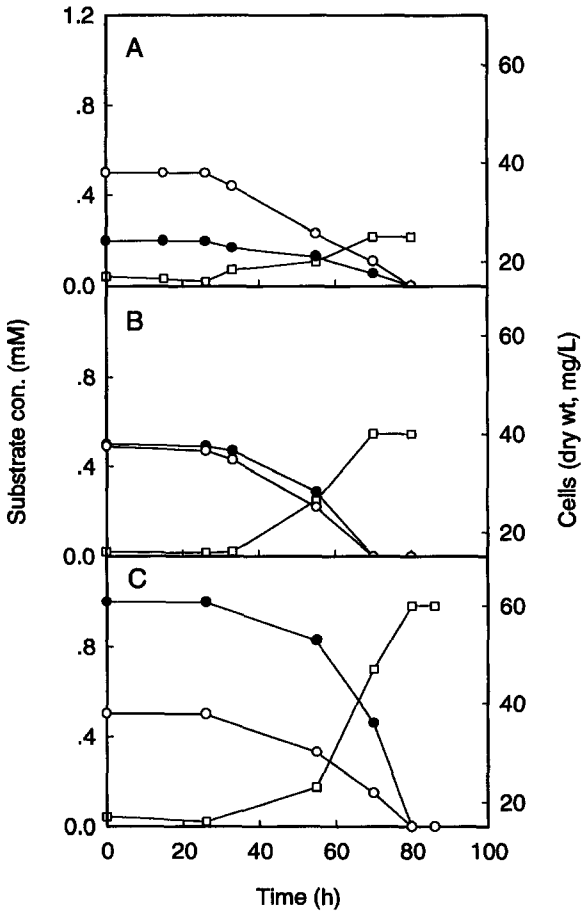

Figure 5. Effect of initial phenol concentrations on the simultaneous degradation of phenol and 4-CP in batch cultures. Symbols: ○, 4-CP; ●, phenol; □, cell growth.

Table 1. Specific enzyme activities in cell extracts of CPW301

Enzyme assayed and assay substrate	Specific activities[a] : Growth substrate		
	Glucose	Phenol	4-CP
Catechol 2,3-dioxygenase			
Catechol	< 0.001	0.171	0.169
3-Methylcatechol	< 0.001	0.027	0.035
4-Methylcatechol	< 0.001	0.064	0.043
4-Chlorocatechol	< 0.001	0.030	0.018
Catechol 1,2-dioxygenase			
Catechol	< 0.001	0.001	< 0.001
4-Methylcatechol	< 0.001	0.001	< 0.001
4-Chlorocatechol	< 0.001	0.001	< 0.001

[a] Activities were measured with the crude extracts of CPW301 cultured with glucose, phenol and 4-CP, respectively.

tration increased the amount of cell mass in the culture. However, the specific 4-CP degradation rate during the exponential growth was about 0.45 mM/(h·g cell) regardless of the change of phenol concentration from 0 to 0.5 mM. When the initial phenol concentration was increased to 1.0 mM, the specific 4-CP degradation rate decreased to 0.31 mM/(h·g cell). Therefore, the time required for the 4-CP degradation could not be shortened further with the increased cell mass.

Discussion

The newly isolated strain CPW301 could fully degrade 4-CP as indicated by the fact that the stoichiometric amount of chloride was accumulated in culture fluids. During the break-down of 4-CP, the *meta*-cleavage product of 4-chlorocatechol, 5-chloro-2-hydroxy muconic semialdehyde, was released as a temporary metabolic intermediate. The *meta*-cleavage products of chlorocatechols were reported to be lethal or unable to support growth of the microorganisms tested (Bartels et al. 1984; Wieser et al. 1994). However, the strain CPW301 could utilize 4-CP as a sole growth substrate via a *meta*-cleavage pathway. Recently, 4-chlorocatechol has been reported to be mineralized via a *meta*-cleavage pathway by some chloroaromatics utilizing strains (Arensdorf & Focht 1995; Hollender et al. 1994).

The strain CPW301 could also degrade phenol. At the same substrate concentration, phenol was degraded much faster than 4-CP. For example, the complete degradation of 0.6 mM 4-CP took about 160 h, whereas that of 0.6 mM phenol took about 24 h (interpolated value). The cells also showed higher growth yield in the presence of phenol than that in the presence of 4-CP. The faster degradation and the higher growth yield with phenol may be due the facts that:

- catechol 2,3-dioxygenase degrades catechol (a phenol metabolite) more effectively than it does 4-chlorocatechol (a 4-CP metabolite); and
- the breakdown of phenol does not produce any detectable intermediates, which allows more carbons in phenol to be utilized for the construction of cell constituents.

Washed cells experiments (Figure 3) showed that both 4-CP- and phenol-grown cells could degrade 4-CP and phenol completely, indicating that the initial part of both degradation pathways of 4-CP and phenol are identical. Enzyme tests revealed that the degradation of both compounds occurred via an inducible *meta*-cleavage pathway (Table 1). Since the initial degradation pathway for phenol and for 4-CP is the same, CPW301 can simultaneously degrade both phenol and 4-CP as a mixture. Furthermore, the addition of phenol increased the cell growth and shorten the time required for the 4-CP degradation. However, the phenol addition did not increase the specific 4-CP degradation rate. These results suggested that the shortened 4-CP degradation time was due to the increased cell mass rather than by the increased degradation capability of the cells.

The addition of phenol did not relieve the 4-CP toxicity. Regardless of phenol addition, the degradation activity was completely inhibited when the initial concentration of 4-CP increased to 0.9 mM (Figures 1 and 4).

It is also noteworthy that the time required for the complete degradation of 4-CP and phenol was exactly the same whether the initial concentrations of 4-CP and phenol were the same or different (Figure 5). This result indicates that both substrates share the same metabolic pathway, and enzymes involved in this pathway have no preference for any one of the two substrates present at the same time. The uptake of both substrates without any preference was even more obvious when the concentrations of both substrates are the same (Figure 4 and Figure 5B).

As a conclusion, a newly isolated strain, *Comamonas testosteroni* CPW301, can degrade 4-CP and phenol mixtures completely via a *meta*-cleavage pathway, which is useful not only for enhanced cell growth but also for the bioremediation of both compounds, which are normally both present at hazardous waste sites.

Acknowledgment

This work was supported by a grant from the Ministry of Science and Technology, Korea.

References

Arensdorf JJ & Focht DD (1995) A *meta*-cleavage pathway for 4-chlorobenoate, an intermediate in the metabolism of 4-chlorobiphenyl by *Pseudomonas cepacia* P166. Appl. Environ. Microbiol. 61: 443–447

Asturias JA & Timmis KN (1993) Three different 2,3-dihydroxybiphenyl-1,2-dioxygenase genes in the gram-positive polychlorobiphenyl-degrading bacterium *Rhodococcus globerulus* P6. J. Bacteriol. 175: 4631–4640

Balfanz J & Rehm H-J (1991) Biodegradation of 4-chlorophenol by adsorptive immobilized *Alcaligenes* sp. A7-2 in soil. Appl. Microbiol. Biotechnol. 35: 662–668

Bartels I, Knackmuss H-J & Reineke W (1984) Suicide inactivation of catechol 2,3-dioxygenase from *Pseudomonas putida* mt-2 by 3-chlorocatechols. Appl. Environ. Microbiol. 47: 500–505

Boyd SA & Shelton DR (1984) Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. Appl. Environ. Microbiol. 47: 272–277

Cole JR, Cascarelli AL, Mohn WW & Tiedje JM (1994) Isolation and characterization of a novel bacterium growing via reductive dehalogenation of 2-chlorophenol. Appl. Environ. Microbiol. 60: 3536–3542

Dorn E & Knackmuss H-J (1978) Chemical structure and biodegradability of halogenated aromatic compounds; substituent effects on 1,2-dioxygenation of catechol. Biochem. J. 174: 85–94

Hollender J, Dott W & Hopp J (1994) Regulation of chloro- and methylphenol degradation in *Comamonas testosteroni* JH5. Appl. Environ. Microbiol. 60: 2330–2338

Feist CF & Hegeman GD (1969) Phenol and benzoate metabolism by *Pseudomonas putida*: regulation of tangential pathway. J. Bacteriol. 100: 869–877

Knackmuss H-J & Hellwig M (1978) Utilization and cooxidation of chlorinated phenols by *Pseudomonas* sp. B13. Arch. Microbiol. 117: 1–7

Lee S-T, Lee S-B & Park Y-H (1991) Characterization of a pyridine-degrading branched gram-positive bacterium isolated from the anoxic zone of an oil shale column. Appl. Microbiol. Biotechnol. 35: 824–829

Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Madsen T & Aamand J (1992) Anaerobic transformation and toxicity of trichlorophenols in a stable enrichment culture. Appl. Environ. Microbiol. 58: 557–561

Menke B & Rehm H-J (1992) Degradation of mixtures of monochlorophenols and phenol as substrates for free and immobilized cells of *Alcaligenes* sp. A7-2. Appl. Microbiol. Biotechnol. 37: 655–661

Mikesell MD & Boyd SA (1986) Complete reductive dechlorination and mineralization of pentachlorophenol by anaerobic microorganisms. Appl. Environ. Microbiol. 52: 861–865

Murray K, Duggleby CJ, Sala-Trepat JM & Williams PA (1972) The metabolism of benzoate and methylbenzoates via the *meta*-cleavage pathway by *Pseudomonas arvilla* mt-2. Eur. J Biochem. 28: 301–310

Smibert RM & Krieg NR (1981) General characterization. In: Gerhardt P, Murry RGE, Costilow R, Nester E, Wood WA, Krier NR & Phillips GB (Eds) Manual of Methods for General Bacteriology (pp 411–423). American Society for Microbiology, Washington, DC

Sondossi M, Sylvestre M & Ahmad D (1992) Effects of chlorobenzoate transformation on the *Pseudomonas testosteroni* biphenyl and chlorobiphenyl degradation pathway. Appl. Environ. Microbiol. 58: 485–495

Tamaoka J & Komagata K (1984) Determination of DNA base composition by reverse-phase high-performance liquid chromatograph. FEMS Microbiol. Lett. 25: 125–128

Tamaoka J, Ha D-M & Komagata K (1987) Reclassification of *Pseudomonas acidovorans* den Dooren de Jong 1926 and *Pseudomonas testosteroni* Marcus and Talalay 1956 as *Comamonas testosteroi* comb. nov., with an emended description of the genus *Comamonas*. Int. J. Syst. Bacteriol. 37: 52–59

Westmeier F & Rehm H-J (1987) Degradation of 4-chlorophenol in municipal wastewater by adsorptive immobilized *Alcaligenes* sp. A 7-2. Appl. Microbiol. Biotechnol. 26: 78–83

Wieser M, Ebenspächer J, Vogler B & Lingens F (1994) Metabolism of 4-chlorophenol by *Azobacter* sp. GP1: structure of the *meta* cleavage product of 4-chlorocatechol. FEMS Microbiol. Lett. 116: 73–78

Woods SL, Ferguson JF & Benjamin MM (1989) Characterization of chlorophenol and chloromethoxybenzene biodegradation during anaerobic treatment. Environ. Sci. Technol. 23: 62–68