Korean J. Soil Sci. & Fert.(2000)
Vol. 33(4) : 261~265

Isolation and Identification of Pentachlorophenol-degrading Bacteria

Young-Doo Park and Jin-Seong Eum

ABSTRACT

To develope the enhanced bacterial strains capable of biodegradation for various chlorinated aromatic compounds, 100 bacterial strains were isolated from soil samples of suburbs of Taejon, Cheongju, and Jeonju by the enrichment culture. These strains can degrade pentachlorophenol (PCP) which is a kind of wood preservatives. Nineteen strains of the isolates were selected by fast colony-forming rate on solid minimal media containing PCP as an only source of carbon and energy. These strains were identified to genus level. Fifteen strains were identified as *Pseudomonas*, 1 strain as *Acinetobacter* and 3 strains were not. Genus *Alcaligenes* strains were not found among them. *Pseudomonas* sp. MU135. MU139, MU163 and MU 184 were able to degrade for 4 kinds of chlorinated compounds, PCP, 2,4-D, MCPA and 3CB. *Pseudomonas* sp. If was observed that MU139 exhibits the highest degradability in liquid minimal media at 72 hours after inoculation. *Pseudomoans* sp. MU147, MU177, MU184 and MU192 also degraded the compounds at higher rates. As the results, Pseudomonas sp. MU139 and unidentified strain MU184 had biodegrability for broad range of chlorinated compounds and higher rates of degradation for PCP.
Key words : PCP, Recalcitrants, Biodegradability, Taejon.

Introduction

Man-made chemicals used as solvents, pesticides and herbicides, etc., cause considerable environmental pollution and human health problems as a result of their persistence, toxicity, and transformation into hazardous metabolites.

Pentachlorophenol(PCP) and its sodium salt (Na-PCP) are used throughout the world as biosides. Most PCP is used by the wood preserving industry, but significant quantities are used in herbicides, algicides, molluscicides and disinfectants(Kauffman, 1977). PCP appears to accumulate within food chains(Lee *et al.*, 1977) and is thought to be mutagenic or at least comutagenic(Dougherty, 1977). Environmental contamination by PCP is wide spread. Of particular concern is contamination of soil, especially near wood-treatment facilities. Such contamination often threatens groundwater supplies, as PCP readily leaches through porous soils into aquifers.

As is typical of most chlorinated pesticides, PCP is somewhat resistant to biodegradation. However, the ability to degrade PCP has been demonstrated by a variety of pure cultures of microorganisms(Pignatello *et al.*, 1983; Watanabe, 1973). Work by Edgehill and Finn(1982) and Pignatello *et al.*(1983) suggests that such microorganisms might be useful for removal of PCP from contaminated soils and waters.

* Faculty of Life Science, Mokwon university, Taejeon 302-318, Korea

In order to develop the enhanced bacterial strains, here we isolated PCP-degrading bacterial strains and identified strains selected with faster degradability.

Materials and Methods

1. Habitats.

Five habitats were sampled for the presence of PCP-degrading microorganisms. The areas were Sintanjin, Daehwadong of Taejon, 1,2 industrial complex, and 3,4-industrial complex of Cheongju, and Palbokdong of Jeonju. Factories of lumber industry were located there.

2. Media

All isolation step were performed in media with a mineral salts (MS) base of the following composition (in grams per liter): K_2HPO_4, 1.73; K_2HPO_4, 0.68; NH_3NO_3, 1.0; $MgSO_4 \cdot 7H_2O$, 0.03; and $FeSO_4 \cdot 7H_2O$, 0.03. The calcium, manganese, and iron salts were made up separately as a 100 concentrate, acidified, filter sterilized, and added after autoclaving. The final pH of the MS base was adjusted to 7.4 with ammonium hydroxide.

PCP, when used as the carbon source, was first converted to its sodium salt by being dissolved in 0.2N NaOH. Portions of a stock sodium PCP solution(10,000μg ml^{-1})were then added to MS base, before or after autoclaving, and designated as PCP medium. The concentration of PCP was 40 to 50 μg of PCP per ml.

3. Isolation of procedure.

Samples were inoculated into PCP medium and incubated at 30℃ at 200rpm. Cell growth was monitored by measuring the absorbance at 600 nm, and PCP content was monitored by measuring the absorbance at 320nm (analytical methods). When the PCP content decreased to 1 to 10 μg ml^{-1}, additional stock sodium PCP solution was added to bring the PCP content of the medium back up to 50 μg ml^{-1}. and the maximum concentration of PCP in the medium was raised to 100 μg ml^{-1}. When the culture repeatedly degraded the PCP that was added, a 10%(vol/vol) inoculum was transferred to PCP medium containing 100 μg of PCP per ml as the sole carbon source.

4. Identification of PCP degraders.

The bacterial stains were identified by the Bergey's manual of determinative bacteriology (Holt, 1994). The Procaryotes (Balows *et al.*, 1992) was also referred. Experimental procedures were described below.

4.1. Morphological characteristics

Motility was investigated in mobile media(Edwards and Bruner, 1942) and the number of flagella and their motility were observed by the method of Mayfied and Innis(1997).

4.2. Physiolgical and biochemical characteristics.

The activity of enzyme catalase and oxidase was measured acccording to Gerhardt (1981) and Kovacs (1956). OF(oxidase and Fermentative) test was performed by Hugh and Leifson (1953).

Results and Discussions

1. Identification of selected bacterial strains

One hundred bacterial strains isolated from soil samples of five regions were inoculated on the solid media supplied with pentachlorophenol as a sole source of carbon and energy, and 19 strains showing rapid growth rate and large colonies were selected.

Seven strains making greatest number were selected in soil from Cheongju 3,4-industrial complex, and Palbokdong and Daehwadong followed. These 19 strains were identified to genus level and the results were showed in Table 1 and 2. 15 strains among them were identified as genus *Pseudomonas*, 1 were *Acinetobacter* and 3 strains were not identified. The most strains belonged to genus *Pseudomonas*. No strains were identified as *Alcaligenes*.

Table 1. Characteristics of *Pseudomonas*, *Alcaligenes* and *Acinetobacter*

Characteristics	*Pseudomonas*	*Alcaligenes*	*Acinetobacter*
Shape	Rod	Rod	Coccobacillus
Gram stain	-	-	-
Oxidase	+	+	+
Catalase	+	+	+
OF test	Oxidative	Alkaline	Oxidative
Motility	+	+	+
Flagella	Polar	Lateral	Polar
Spore	-	-	-

Table 2. Identification of the selected bacteria strains

Strain	Area	Genus
MU 134	Daehwadong	*Pseudomonas*
MU 135	Daehwadong	*Pseudomonas*
MU 138	Daehwadong	*Pseudomonas*
MU 139	Daehwadong	*Pseudomonas*
MU 140	Daehwadong	*Pseudomonas*
MU 147	Cheongju 1,2 I.C.*	*Pseudomonas*
MU 149	Cheongju 1,2 I.C.	*Pseudomonas*
MU 163	Cheongju 3,4 I.C.	*Pseudomonas*
MU 165	Cheongju 3,4 I.C.	*Acinetobacter*
MU 168	Cheongju 3,4 I.C.	Unidentified
MU 174	Cheongju 3,4 I.C.	*Pseudomonas*
MU 176	Cheongju 3,4 I.C.	*Pseudomonas*
MU 177	Cheongju 3,4 I.C.	*Pseudomonas*
MU 179	Cheongju 3,4 I.C.	*Pseudomonas*
MU 182	Palbokdong	*Pseudomonas*
MU 184	Palbokdong	Unidentified
MU 187	Palbokdong	*Pseudomonas*
MU 192	Palbokdong	Unidentified
MU 199	Palbokdong	*Pseudomonas*

* I.C. : Industrial Complex

2. Degradability of selected strains for chlorinated aromatics.

Degradative ability of 19 strains selected on solid minimal media were investigatedfor 2,4-dichlorophenoxyacetate(2,4-D), 2-methyl-4-chlorophenoxyacetate(MCPA) and 3-chlorobenzoate(3CB). 2,4-D and MCPA were widely used as herbicides, and 3CB as chemical solvent(Table 3). These are all recalcitrant chlorinated aromatic compounds(Don et al., 1985; Dorn et al., 1978)

Four strains, MU135, MU139, MU140 and MU163, degrade these three compounds and six strains affect two compounds. Seven strains showed no activity for any of them. The selected strains had degradability for chlorinated aromatics in addition to PCP. It was supposed that substrate specificity of the degradative enzymes was relaxed by structural similarity of these chemicals.

Table 3. Degradative activies of selected strains for chlorinated aromatics

Strain	Substrates		
	MCPA	2,4-D	3CB
MU 134	-	-	-
MU 135	+	+	+
MU 138	+	-	+
MU 139	+	+	+
MU 140	+	+	+
MU 147	+	+	-
MU 149	-	-	-
MU 163	+	+	+
MU 165	+	-	+
MU 168	-	-	-
MU 174	-	+	-
MU 176	-	-	-
MU 177	-	-	-
MU 179	-	+	-
MU 182	-	+	+
MU 184	-	+	+
MU 187	-	+	+
MU 192	-	-	-
MU 199	-	-	-

3. Growth rates of selected strains in liquid minimal media.

The selected strains were inoculated into liquid minimal media after the primary selection on solid media and the growth rates were measured by spectrophotometry at 600nm(Table 4).

The optical density of *Pseudomonas* sp. MU139 showed the highest as 0.234 at 72 hours after inoculation. *Pseudomonas* sp. MU147 and MU177, and Unidentified strains MU184 and MU192 grew to about 0.12-0.20. It was investigated that 36-72 hours stage after inoculation into liquid media corresponded to logarithmic phase of growth cycle. The stationary and death phase were not showed. MU139 identified as *Pseudomonas* utilized 4 kinds of chemicals and degraded PCP most rapidly. Unidentified strain MU184 showed degradative ability for 3 kinds of chemicals including

Table 4. Degradative abilities of selected strains in liquid minimal media

Strains	12hr	24hr	36hr	48hr	60hr	72hr
MU 134	0.011	0.011	0.013	0.042	0.065	0.059
MU 135	0.015	0.016	0.016	0.019	0.056	0.055
MU 138	0.018	0.016	0.017	0.027	0.057	0.096
MU 139	0.021	0.025	0.032	0.035	0.067	0.234
MU 140	0.012	0.015	0.014	0.017	0.018	0.012
MU 147	0.015	0.021	0.101	0.176	0.176	0.182
MU 149	0.014	0.006	0.014	0.018	0.011	0.051
MU 163	0.003	0.004	0.010	0.017	0.017	0.136
MU 165	0.007	0.005	0.055	0.091	0.058	0.043
MU 168	0.009	0.010	0.027	0.012	0.022	0.093
MU 174	0.011	0.013	0.078	0.121	0.016	0.082
MU 176	0.003	0.017	0.003	0.008	0.169	0.053
MU 177	0.064	0.011	0.024	0.014	0.037	0.126
MU 179	0.026	0.014	0.003	0.020	0.006	0.014
MU 182	0.011	0.005	0.022	0.050	0.163	0.058
MU 184	0.014	0.003	0.005	0.010	0.028	0.129
MU 187	0.017	0.054	0.026	0.031	0.048	0.048
MU 192	0.022	0.012	0.016	0.033	0.022	0.209
MU 199	0.001	0.021	0.016	0.015	0.002	0.010

PCP with relatively high rate. The research for genetic and physiological characteristics of these strains will be available to develop the enhanced bacterial strains with powerful degradability for various recalcitrant compounds.

References

Balows, A., H. G. Trueper, M. Dworkin, W. Harder, and K-H Schleifer(Edt), 1992. The Prokaryotes, Springer-Verlag. Evance, W. C., B. S. W. Smith, P. Mose and H. N. Fernley, 1971. Bacterial metabolism of 4-chlorophenoxy acetate. Biochem. J. 122, 509-517.

Don, R. H., A. J. Weightmann, H. J. Knackmuss, and K. N. Timmis, 1985. Transposon mutagenesis and cloning analysis of the pathways for degradation of 2,4- dichloro- phenoxyacetic acid and 3-chlorobenzoate in *Alcaligenes eutrophus* JMP 134(pJP4). J. Bacteriol. 161, 85-90

Dorn, E., Hellwig, W. Reineke, and H. J. Knackmuss, 1978. Chemical structure and biodegradation of chlorinated aromatic compounds. Biochem. J. 174, 73-84.

Dougherty, R. C. in *Pentachlorophenol: Chemistry, Pharmacology and Enwironmaental Toxicology* Plenum Press Inc., New York, 1977, pp. 351-361

Edgehill. R. U., and R. K. Finn. 1982. Isolation, characterization and growth kinetics of bacteria metabolizing pentachlorophenol. Eur. J. Appl. Microbiol. Biotechnol. 16:179-184

Edwards, D. R. and D. W. Bruner, 1942. Serological identification of *Salmonella* culture. circ. ky. agric. Exp. Sta. 110, 54-66.

Gerhardt, P., R.G.E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krig, and G.B. Phillips, 1981. Manual of methods for general bacteriology, American society for microbiology.

Higgins, I. J. and R. G. Burns. 1975. The chemistry and microbiology of pollution. Academic press.

Hugh, R. and E. Leifson, 1953. The taxomomic significance of fermentative versus oxidative metabolism of carbohydrate by various Gram-negative. J. Bacterol. 66, 24-26.

Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams, 1994. Bergey's manual of determinative bacteriology. Williams & Wilkins.

Kaufman, D. D.1997 In *Pentachlorophenol: Chemistry, Pharmacology and Enwironmental Toxicology* Plenum Press Inc., New York, 1977, pp. 27-39

Kovacs, N. 1956. Identification of *Pseudomonas pyocenea* by the oxidase reaction. Nature. 178, 703.

Lee, P., Metcalf, R. L. and L. K. 1997 In *Pentachlorophenol: Chemistry, Pharmacology and Enwironmaental Toxicology* Plenum Press Inc., New York, 1977, pp 53-63

Mayfield, C. I. and W.E. Innis, 1977. A rapid method for staining bacterial flagella. Can. J. Microbiol., 23, 1311-1313.

Pignatello, J. J., M. M. Martinson, J. G. Steiert, R. E. Carsion, and R. L. Crawford. 1983. Biodegradation and photolysis of pentachlorophenol inartificial freshwater streams. Appl. Environ. Microbiol. 46:1024-1031.

Watanabe, I. 1973. Isolation of pentachlorophenol decomposing bacteria from soil. Soil Sci. Plant Nutr. 19:109-116

韓土肥誌(2000)
Vol. 33(4) : 261~265

Isolation and Identification of Pentachlorophenol-degrading Bacteria

Young-Doo Park and Jin-Seong Eum

Pentachlorophenol을 분해하는 세균의 분리와 동정

박영두 · 음진성

공해 문제를 유발하고 있는 Pentachlorophenol(PCP)와 2,4-dichlorophenoxyacetate(2,4-D)와 같은 난분해성의 염소계 방향족 화합물들을 폭 넓고 빨리 분해할 수 있는 우수 균주를 개발하기 위한 기초 연구로서, 대전, 청주 ,전주 근교 지역에서 채취한 토양 표품으로부터 100균주의 세균을 분리하였다. 그 중 PCP를 단일 탄소와 에너지원으로 하는 고체 최소 배지에서 잘 자라는 균주 19균주를 선별하였다. 이들 균주를 동정한 결과 *Pseudomonas*속이 15균주, *Acinetobacter* 속이 1균주, 3균주는 미동정되어 *Pseudomonas*속이 대부분을 차지하였다. 분해능이 비교적 좋은 것으로 알려진 *Alcaligenes*속의 세균은 발견되지 않았다.

*Pseudomonas*속으로 밝혀진 MU135, MU139, MU163과 MU184 네가지 염소계 화합물들(PCP, 2,4,-D, MCPA 그리고 3CB)을 모두 분해하는 것으로 나타났다. 최소 액체 배지에서 배양한 경우 *Pseudomonas* sp. MU139 균주가 접종 72시간 후에 가장 높은 분해도를 나타내었고, *Pseudomonas* sp. MU147, MU177, MU184 그리고 MU192는 그 다음으로 높은 분해도를 나타냈다. 실험결과 선별된 19 균주 중 *Pseudomonas* sp. MU139와 *Pseudomonas* sp. MU184는 염소계 방향족 화합물에 대한 넓은 분해능을 갖고 있으며, 특히 PCP에 대한 높은 분해도를 나타내는 것으로 조사되어 우수 균주 개발의 좋은 재료로 이용될 수 있을 것으로 보인다.

Key words : PCP, Recalcitrants, Biodegradability, Taejon.

* 목원대학교(Faculty of Life Science, Mokwon university, Taejeon 302-318, Korea)