Appl Microbiol Biotechnol (2001) 55:248–253
DOI 10.1007/s002530000500

ORIGINAL PAPER

Hiroyuki Futamata · Shigeaki Harayama
Kazuya Watanabe

Diversity in kinetics of trichloroethylene-degrading activities exhibited by phenol-degrading bacteria

Received: 2 June 2000 / Accepted: 15 August 2000 / Published online: 7 December 2000

Abstract Whole-cell kinetics of phenol- and trichloroethylene (TCE)-degrading activities expressed by 13 phenol-degrading bacteria were analyzed. The K_s (apparent affinity constant in Haldane's equation) values for TCE were unexpectedly diverse, ranging from 11 µM to over 800 µM. The V_{max}/K_s values for phenol were three orders of magnitude higher than the values for TCE in all bacteria analyzed, suggesting that these bacteria preferentially degrade phenol rather than TCE. A positive correlation between K_s for phenol and K_s for TCE was found, i.e., bacteria exhibiting high K_s values for phenol showed high K_s values for TCE, and vice versa. A comparison of the K_s values allowed grouping of these bacteria into three types, i.e., low-, moderate- and high-K_s types. Pseudo-first-order degradation-rate constants for TCE at 3.8 µM were found to be adequate to rapidly discriminate among the three types of bacteria. When bacteria were grown on phenol at the initial concentration of 2 mM, *Comamonas testosteroni* strain R5, a representative of low-K_s bacteria, completely degraded TCE at 3.8 µM, while strain P-8, a representative of high-K_s bacteria, did not. A mixed culture of these two bacteria poorly degraded TCE under the same conditions, where P-8 outgrew R5. These results suggest that low-K_s bacteria should be selectively grown for effective bioremediation of TCE-contaminated groundwater.

Introduction

Contamination of subsurface environments with chlorinated hydrocarbons, in particular trichloroethylene (TCE) and perchloroethylene, is a potentially serious threat to drinking-water sources. A number of laboratory studies have demonstrated that these compounds are transformed cometabolically by aliphatic and aromatic hydrocarbon-degrading bacteria (Ensley 1991; Semprini 1997). In addition, several field trials of TCE bioremediation have been reported. These trials include injection of methane (Semprini et al. 1990, 1991), toluene (Hopkins and McCarty 1995; McCarty et al. 1998) and phenol (Hopkins et al. 1993; Hopkins and McCarty 1995) to stimulate endogenous bacteria. Another type of bioremediation includes the introduction of exogenous TCE-degrading bacteria (Duba et al. 1996). Although injection of methane has been carried out most frequently, Hopkins et al. (1993) have documented that phenol injection was more effective in TCE removal than methane injection. Fries et al. (1997) compared the effects of phenol and toluene on microbial populations in a TCE-contaminated aquifer; and they have suggested that phenol may be a better cosubstrate than toluene, due to its lower toxicity. These studies have proposed phenol to be an effective cosubstrate for TCE bioremediation, although phenol addition may be questioned since chlorination of groundwater containing phenol produces chlorinated phenols that cause taste and odor problems (Hopkins et al. 1993).

Laboratory studies have suggested factors that should be considered for the effective bioremediation of TCE. Chu and Alvarez-Cohen (1998) examined the effects of nitrogen sources on the growth of methane-oxidizing bacteria and their TCE degradation. A study with defined, mixed cultures of TCE-transforming and nontransforming toluene-degrading bacteria has suggested that TCE transformers are less competitive due to the accumulation of toxic TCE metabolites in TCE-transforming cells (Mars et al. 1998). Concentrations of toluene (Mu and Scow 1994; Fries et al. 1997) and phenol (Shih et al. 1996; Fries et al. 1997) supplied to culture media have also been examined in respect to TCE degradation. These studies have suggested that concentrations of these compounds exert large effects on microbial populations and the cometabolic activities. Competition for the enzyme between phenol and TCE has been discussed according to the whole-cell kinetics exhibited by

H. Futamata (✉) · S. Harayama · K. Watanabe
Marine Biotechnology Institute, Kamaishi Laboratories,
3-75-1 Heita, Kamaishi City, Iwate 026-0001, Japan
e-mail: hiroyuki.futamata@kamaishi.mbio.co.jp
Tel.: +81-193-265781, Fax: +81-193-266592

Pseudomonas cepacia G4 (Folsom et al. 1990). In that study, the K_s (apparent affinity constant in Haldane's equation) value for TCE (3 μM) was determined to be lower than that for phenol (8.5 μM), although the authors encountered a technical limitation in the measurement of phenol-degrading activity at low concentrations (Folsom et al. 1990). It is considered that substrate interaction is quite important when applying bacterial cometabolic activities to TCE bioremediation.

In this study, we analyzed the whole-cell kinetics of phenol- and TCE-degrading activities expressed by phenol-degrading bacteria. One aim was to understand more accurately the competitive interaction between phenol and TCE in phenol-degrading bacteria. Since determination of the K_s value for phenol requires the measurement of activity at low concentrations (below 10 μM), the phenol oxygenation-dependent oxygen-consumption assay (Watanabe et al. 1996) was employed, rather than the phenol-disappearance assay of Folsom et al. (1990). Another aim was to obtain insights into the diversity of phenol-degrading bacteria in TCE degradation kinetics. Implications of these kinetic data for in situ TCE bioremediation are discussed.

Materials and methods

Bacterial strains

Bacterial strains used in this study are listed in Table 1. Strains E1, E6, R2 and R5 were isolated from an enrichment formed in a chemostat culture grown on phenol (Watanabe et al. 1996). In contrast, strains BH (Hashimoto and Fujita 1987), CF600 (Frey et al. 1983), P-2, P-5, P-6, P-8 and P-10 (Futamata et al. 1998), P35X (Hopper et al. 1970) and WAS2 (Watanabe et al. 1996) were isolated from enrichments in batch cultures grown on phenol or substituted phenols. Strains P-2, P-5, P-6, P-8 and P-10 were tentatively identified according to their 16S ribosomal DNA and *gyrB* (Yamamoto and Harayama 1998) sequences.

TCE- and phenol-degrading activities

Bacterial strains were grown at 25 °C in BSM medium (Atlas 1993) supplemented with 2 mM phenol. The medium contained 12.5 g K_2HPO_4, 3.8 g KH_2PO_4, 1.0 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4{\cdot}7H_2O$ and 5 ml of trace-element solution per liter (pH 7.2). The trace-element solution contained 0.232 g H_3BO_3, 0.174 g $ZnSO_4{\cdot}7H_2O$, 0.116 g $FeSO_4(NH_4)_2SO_4{\cdot}6H_2O$, 0.096 g $CoSO_4{\cdot}7H_2O$, 0.022 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 8 mg $CuSO_4{\cdot}5H_2O$ and 8 mg $MnSO_4{\cdot}4H_2O$ per liter. Cells were harvested at the early- or mid-exponential growth phase by centrifugation at 4 °C for 15 min at 10,000 *g*, washed with 10 ml of 10 mM potassium phosphate buffer at pH 7.0 (KP) and resuspended in KP with an optical density at 600 nm of 1.5–2.0. A dry cell weight in a cell suspension was determined gravimetrically by filtration though a 0.22-μm pore-size membrane. One milliliter of the cell suspension and 4 ml of KP containing various concentrations of TCE were put in a bottle (33 ml capacity) which was then capped with a Teflon-lined rubber septum and sealed with an aluminum crimp seal. The bottle was shaken at 120 rpm at 25 °C and the TCE concentration was determined at appropriate intervals, as described below. A TCE-degrading activity at each concentration was estimated from an initial decrease in the TCE concentration.

A phenol-degrading activity was calculated from a phenol oxygenation-dependent oxygen-consumption rate (phenol-oxygenat-

Table 1 Kinetic parameters in Haldane's equation for phenol and trichloroethylene (TCE) degradation. See text for full definition of each parameter

Strain	Apparent kinetic parameters for TCE			Apparent kinetic parameters for phenol			
	K_s (μM)	K_I (μM)	V_{max} (μmol min^{-1} g^{-1} dry cells)	V_{max}/K_s (l min^{-1} g^{-1} dry cells)	K_s (μM)	K_I (μM)	V_{max} (μmol min^{-1} g^{-1} dry cells)	V_{max}/K_s (l min^{-1} g^{-1} dry cells)
Pseudomonas putida P-2	590±220	>1,600	3.9±1.0	0.0070	4.1±1.2	3,100±1300	13±1.1	4.2
P. putida P-5	>800	>1,600	>6.7	0.0080	2.9±0.7	1,100±260	48±2.8	16.0
P. putida P-6	790±440	>1,600	5.6±1.8	0.0070	3.9±1.7	460±260	16±2.9	6.0
P. putida P-8	820±270	>1,600	4.2±0.7	0.0050	5.3±1.9	6,900±1600	23±1.2	3.0
P. putida P35X	120±33	740±330	0.5±0.1	0.0050	3.6±1.3	9,300±2100	91±3.8	13.0
P. putida BH	260±60	>250	0.3±0.08	0.0030	3.2±0.4[a]	2,800±760[a]	20±1.5[a]	5.7
Pseudomonas sp. CF600	180±160	>1,600	1.5±0.4	0.0080	3.0±0.4[a]	2,900±850[a]	9±1.0[a]	3.0
Pseudomonas sp. WAS2	360±35	>1,000	0.9±0.03	0.0020	2.2±0.5[a]	3,300±670[a]	14±0.5[a]	6.3
Ralstonia sp. P-10	59±12	>270	0.6±0.04	0.010	4.4±2.0	110±60	100±19	23.0
Burkholderia sp. E1	23±6.8	>130	1.2±0.2	0.054	0.5±0.1[a]	210±40[a]	50±2.5[a]	100.0
Comamonas testosteroni E6	32±6.5	>550	0.7±0.04	0.020	0.1±0.04[a]	170±30[a]	40±2.0[a]	360.0
C. testosteroni R2	35±13	>350	1.3±0.2	0.037	0.9±0.1[a]	240±30[a]	130±4.0[a]	140.0
C. testosteroni R5	11±2.5	>550	1.0±0.1	0.091	0.4±0.1[a]	120±20[a]	270±12[a]	730.0

[a] These data are cited from Watanabe et al. (1996)

ing activity) according to the method described previously (Watanabe et al. 1996). A cell suspension was infused into a cuvette (2 ml) of a Clark-type oxygen electrode (5/6 Oxygraph, Gilson, Middleton, USA) containing air-saturated BSM medium at 25 °C. After the respiratory oxygen consumption was inhibited by adding potassium cyanide (10 mM), phenol was added to measure the phenol-oxygenating activity. The phenol-degrading activity was estimated as half of the phenol-oxygenating activity, since two oxygen molecules are consumed per mole of phenol in the *meta*-cleavage pathway (Watanabe et al. 1996). All strains used in this study produced yellow pigments when growing on phenol, indicating that they degrade phenol through the *meta*-cleavage pathway (Harayama et al. 1992).

A pseudo-first-order degradation-rate constant (k_1) for TCE was determined at a concentration of 3.8 µM at 25 °C, as described by Speitel Jr et al. (1993). The following equation was used for the calculation:

$$k_1(\text{l g}^{-1}\ \text{h}^{-1}) = -[1+H\times(V_G/V_L)]\times\ln(C_t/C_0)/(X\times t)$$

where C_0 and C_t are the TCE concentrations (grams per liter) in the head-space gas at incubation times 0 and t respectively, X is the dry cell weight (grams per liter), H is the dimensionless Henry constant at 25 °C (0.415), V_G is the gas volume and V_L is the liquid volume.

Kinetic analysis

Kinetic analyses of TCE- and phenol-degrading activities were conducted according to Haldane's equation (Yang et al. 1975; Folsom et al. 1990)

$$v=[V_{max}\times(S)]/[(S)+K_s+(S)^2/K_I]$$

where v is the degradation rate, (S) is the substrate concentration, K_s is the half-saturation constant, K_I is the inhibition constant, and V_{max} is the theoretical maximum activity. The activities at more than ten different substrate concentrations were used to calculate these kinetic parameters, using JMP statistical visualization software (SAS Institute, Cary, N.C., USA). Following Folsom et al. (1990), the term K_s was employed instead of K_m, because the velocity was measured by using intact cells and not enzymes. Values are described as apparent because no attempt was made to vary the concentration of oxygen or other cosubstrates.

Degradation of TCE under growing conditions

One-tenth strength tryptic soy broth (1/10 TSB) medium (Difco, Detroit, USA) was inoculated with cells on an agar plate and shaken at 100 rpm for 26 h at 25 °C. A sample (0.2 ml) of the culture was transferred to 20 ml of fresh 1/10 TSB and shaken at 100 rpm for 14 h at 25 °C. The cells were collected by centrifugation, washed with BSM medium and resuspended at 3.0×10^5 cells ml^{-1} in BSM medium amended with 2 mM phenol. The cell suspension (20 ml) was put in a bottle (120 ml capacity) sealed with a Teflon-butyl septum. TCE was added by microsyringe through the septum at the initial concentration of 3.8 µM. The bottle was shaken at 100 rpm at 25 °C. Cell numbers (colony forming units) in each culture were determined using agar plates containing 1/10 TSB medium. In a mixed culture, the initial cell concentration of each bacterium was adjusted to 3.0×10^5 cells ml^{-1}. Strains R5 and P-8 could be separately counted on 1/10 TSB plates according to their different colony morphologies.

Analytical methods

TCE was measured using a gas chromatograph (the GC-15 A system; Shimadzu, Kyoto, Japan) equipped with a 30-m megabore fused silica capillary column (DB-624; J&W Scientific, Folsom, USA) and a hydrogen flame ionization detector. Samples of vapor (200 µl) were taken from each crimp-sealed bottle using a microsyringe and injected into the gas chromatograph. Operating conditions were as follows: injector temperature 120 °C, detector temperature 160 °C, oven temperature 60 °C and the flow rate of carrier nitrogen gas was 30 ml min^{-1}. Under these conditions, TCE was detected at a retention time of 1.8 min. TCE concentration was determined by comparing each peak area with that of pure reagent-grade TCE.

Phenol concentration was measured using a high-pressure liquid chromatograph (Toso, Tokyo, Japan) equipped with a STV-80 column (Toso) and a UV detector. Eluates were monitored at 269 nm. Phenol was eluted by increasing the methanol concentration in water; and the concentration gradient program consisted of 30% for 5 min, a linear gradient of 30–90% for 5 min and then 90% for 10 min. Phenol was identified according to the retention time and the concentration was determined by comparing a peak area with that of pure reagent-grade phenol.

Accession number

The nucleotide sequences reported in this paper have been deposited in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases under accession nos. AB038135 through AB038143.

Results

Whole-cell kinetics of TCE- and phenol-degrading activities

Typical examples of the correlations between TCE concentration and TCE-degrading activity expressed by phenol-degrading bacteria are shown in Fig. 1. We applied Haldane's equation to analyze the kinetics, as its utility for analyzing bacterial phenol-degrading activity has been demonstrated (Folsom et al. 1990; Watanabe et al. 1996). When the substrate-dependent inhibition of the degradation rate is very weak (i.e., the K_I value is very large), a term in Haldane's equation [$(S)^2/K_I$] becomes negligible, allowing Haldane's equation to become almost identical to the Michaelis–Menten equation. Kinetic constants in Haldane's equation for TCE- and phenol-degrading activities of 13 phenol-degrading bacteria were determined (Table 1). We could not estimate reliable K_I values for TCE degradation by most bacterial strains, since TCE-degrading activities did not sufficiently decrease, even up to the highest concentration tested, i.e., 1,600 µM (Fig. 1). The K_s values for TCE were unexpectedly diverse ranging from 11 µM to over 800 µM. A V_{max}/K_s value has been recognized as an important index to evaluate enzymatic reactions (Fersht 1977); the values for phenol were three orders of magnitude higher than the values for TCE in all the bacteria analyzed (Table 1).

A positive correlation between K_s for phenol and K_s for TCE was found (Fig. 2), i.e., bacteria exhibiting high K_s values for phenol showed high K_s values for TCE, and vice versa. In addition, phenol-degrading bacteria formed three clusters according to their K_s values, as shown in Fig. 2, i.e., low-K_s bacteria, moderate-K_s bacteria and high-K_s bacteria. The low-K_s bacteria also exhibited low K_I values for both phenol and TCE (Table 1).

Figure 3 presents k_1 values for TCE degradation at a concentration of 3.8 µM, a typical TCE concentration in contaminated groundwater (Hopkins et al. 1993; Hanada et al. 1998). This value has often been used to evaluate

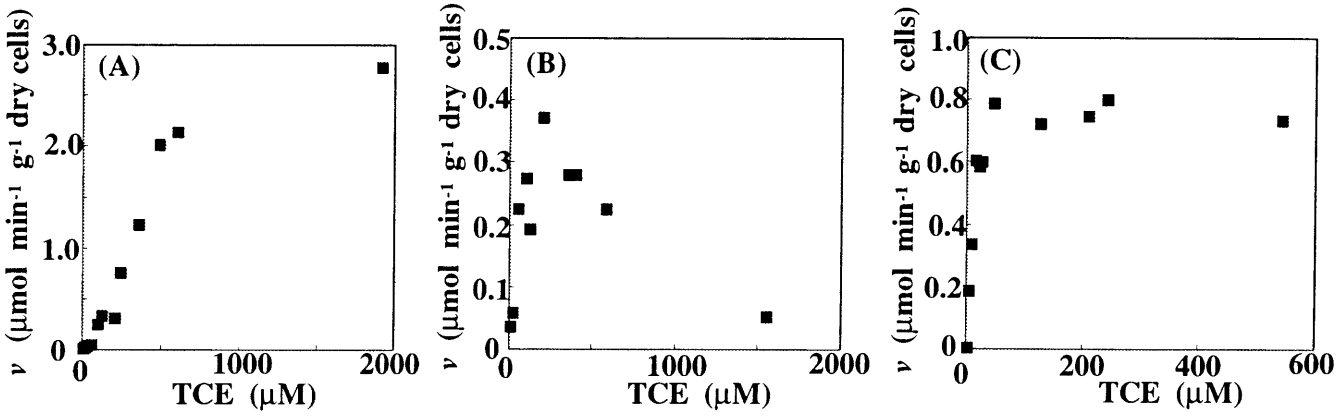

Fig. 1A–C Typical examples of specific trichloroethylene (TCE)-degrading activities determined at various TCE concentrations. **A** Strain P-8, **B** strain P35X and **C** strain R5

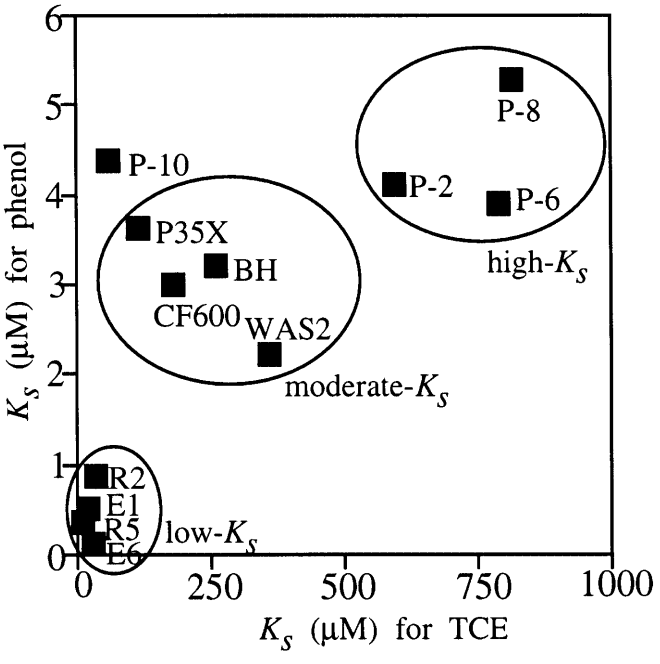

Fig. 2 Correlation between the half saturation constant (K_s) values for phenol and those for TCE. Three clusters are *circled*

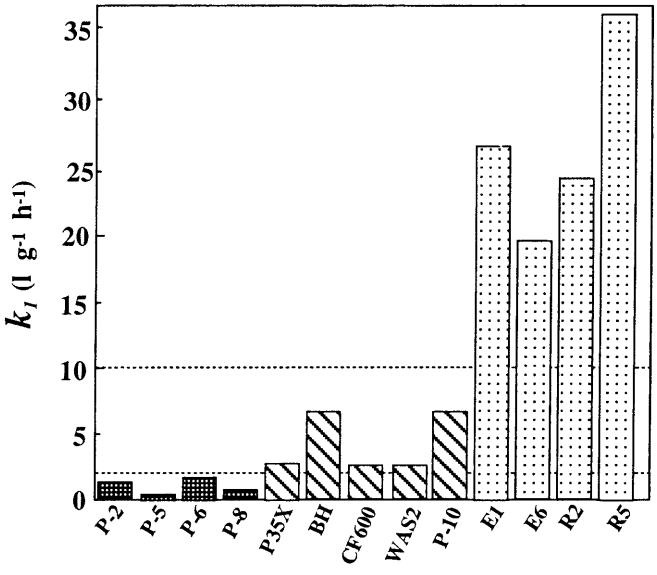

Fig. 3 The pseudo-first-order degradation rate constant (k_1) values for TCE at 3.8 μM of phenol-degrading bacteria. Threshold values are represented by *dotted lines*

TCE-cometabolizing bacteria (Speitel Jr et al. 1993; Hanada et al. 1998). We found that low-K_s phenol-degrading bacteria showed high k_1 values. For instance, the k_1 value of strain R5 was found to be the highest value among aromatic-compound-degrading bacteria so far analyzed (Hanada et al. 1998). We also found threshold values that discriminated k_1 values of low-K_s and moderate-K_s phenol-degrading bacteria (2 l g^{-1} h^{-1}) and k_1 values of moderate-K_s and high-K_s phenol-degrading bacteria (10 l g^{-1} h^{-1}).

TCE degradation under a growing condition

Degradation of TCE at 3.8 μM was examined with strains R5 and P-8 under a condition where phenol (2 mM) was added to promote their growth (Fig. 4). We employed this phenol concentration following the conditions reported in previous studies (Krumme et al. 1993; Hanada et al. 1998). R5 is a representative of low-K_s bacteria, while P-8 is a representative of high-K_s bacteria (Fig. 2). Under the condition employed, P-8 grew more rapidly than R5, although TCE was degraded only in the R5 culture (Fig. 4A, B). In the R5 culture, TCE degradation was initiated after phenol was completely degraded. In a mixed culture of strains R5 and P-8, TCE was not significantly degraded, despite the fact that phenol was completely degraded (Fig. 4C). In this mixed culture, P-8 outgrew R5.

Discussion

A few studies have previously analyzed the kinetics of TCE degradation by phenol-degrading bacteria (Folsom et al. 1990; Ayoubi and Harker 1998), but no study has

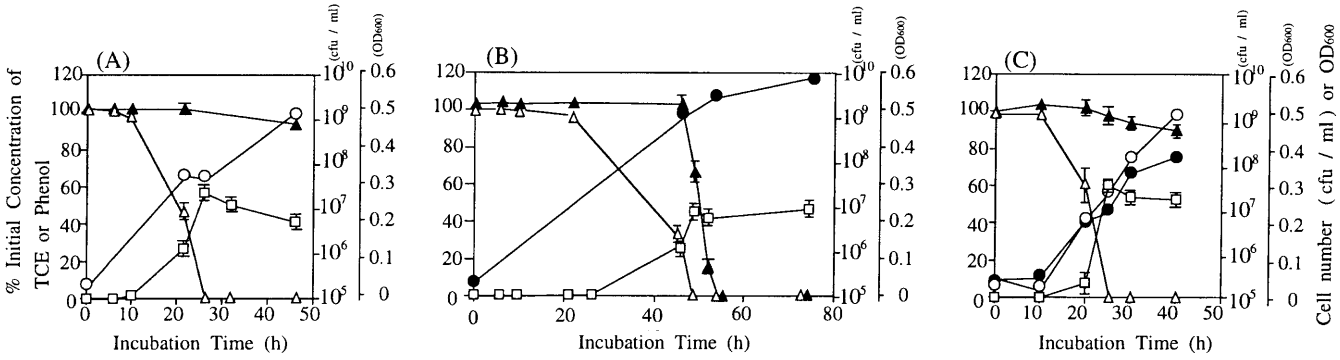

Fig. 4A–C TCE degradation under growing conditions on phenol. **A** Strain P-8, **B** strain R5 and **C** the mixed culture of strains P-8 and R5. ▲ TCE concentration, Δ phenol concentration, □ optical density at 600nm (OD_{600}), ❍ number of P-8 cells and ● number of R5 cells. Each *datum point and error bar* represents the mean ±SE of three different cultures

described comparisons of the kinetic properties of different phenol-degrading bacteria. Since the kinetics for both phenol and TCE degradation were assumed to be essential for understanding TCE bioremediation utilizing the cometabolic activities of phenol-degrading bacteria, this study conducted rigorous comparisons of the kinetic traits. Consequently, our kinetic analyses manifested two competitive behaviors that may be important for understanding TCE bioremediation. First, comparisons of whole-cell kinetics for phenol- and TCE-degrading activities indicated that phenol is a much preferred substrate for the degradative enzymes, rather than TCE, suggesting that TCE is not efficiently degraded in the presence of phenol. This was demonstrated in Fig. 4B.

Second, the data revealed the existence of three types of phenol-degrading bacteria, which exhibited different kinetic properties (Fig. 2). Judging K_s values for TCE, strain G4 was categorized into the low-K_s type (Folsom et al. 1990), while a *Ralstonia eutropha* JMP134 derivative was categorized into the high-K_s type (Ayoubi and Harker 1998). From the k_1 values presented in Fig. 3, low-K_s bacteria were considered to be more useful than the others for TCE bioremediation in groundwater. This idea was demonstrated in Fig. 4, where R5 efficiently degraded 3.8 μM of TCE. In addition, we found a rapid method for discriminating among these different types of bacteria, which employs the k_1 values. This method would be applicable to select phenol-degrading bacteria for bioaugmentation.

The data presented in this study imply that phenol concentration may affect competition between phenol-degrading bacteria exhibiting different kinetic properties for TCE degradation. For instance, high concentrations of phenol may result in inefficient growth of low-K_s bacteria showing high TCE-degrading activities at low TCE concentrations. Actually, the low-K_s bacteria grew more slowly than the high-K_s bacteria, when initial phenol concentrations in the culture media exceeded 1 mM (unpublished data). An example of this growth competition between high-K_s and low-K_s bacteria is shown in Fig. 4C. It is therefore suggested that the phenol concentration should be carefully determined in phenol-amended biostimulation.

In conclusion, this study suggests that low-K_s bacteria should be selectively grown for the effective bioremediation of TCE-contaminated groundwater. To achieve this, specific monitoring of the kinetically different types of phenol-degrading bacteria in TCE bioremediation sites may be necessary. Since whole-cell kinetics of phenol degradation are known to be determined by phenol hydroxylase (Hino et al. 1998), a comparison of the amino acid sequences of their phenol hydroxylases may facilitate the development of genetic markers for their specific detection. The design and evaluation of such genetic markers are underway in our laboratory.

Acknowledgments We thank Sachiko Kawasaki for technical assistance. This work was performed under the management of the Research Institute of Innovative Technology for the Earth as a part of the Research and Development Project on In Situ Soil Bioremediation supported by the New Energy and Industrial Technology Development Organization.

References

Atlas RN (1993) Hand book of microbiological media. CRC Press, Boca Raton, Fla.

Ayoubi PJ, Harker AR (1998) Whole-cell kinetics of trichloroethylene degradation by phenol hydroxylase in a *Ralstonia eutropha* JMP134 derivative. Appl Environ Microbiol 64: 4353–4356

Chu KH, Alvarez-Cohen L (1998) Effect of nitrogen source on growth and trichloroethylene degradation by methane-oxidizing bacteria. Appl Environ Microbiol 64:3451–3457

Duba AG, Jackson KJ, Jovanovich MC, Knapp RB, Taylor RT (1996) TCE remediation using in situ, resting-state bioaugmentation. Environ Sci Technol 30:1982–1989

Ensley BD (1991) Biochemical diversity of trichloroethylene metabolism. Annu Rev Microbiol 45:283–299

Fersht A (1977) Enzyme structure and mechanism. Freeman, New York

Folsom BR, Chapman PJ, Pritchard PH (1990) Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: kinetics and interactions between substrates. Appl Environ Microbiol 56:1279–1285

Frey J, Bagdasarian M, Feiss D, Franklin FCH, Deshusses J (1983) Stable cosmid vectors that enable the introduction of cloned fragments into a wide range of Gram-negative bacteria. Gene 24:299–308

Fries MR, Forney LJ, Tiedje JM (1997) Phenol- and toluene-degrading microbial populations from an aquifer in which successful trichloroethene cometabolism occurred. Appl Environ Microbiol 63:1523–1530

Futamata H, Watanabe K, Harayama S (1998) Relationships between the trichloroethylene-degrading activities and the amino acid sequences of phenol hydroxylases in phenol-degrading bacteria. In: Wickramanayake GB, Hinchee RE (eds) 1st Int Conf Remediation of Chlorinated and Recalcitrant Compounds. Battelle Press, Columbus, Ohio, pp 99-104

Hanada, S., Shigematsu T, Shibuya K, Eguchi M, Hasegawa T, Suda F, Kamagata Y, Kanagawa T, Kurane R (1998) Phylogenetic analysis of trichloroethylene-degrading bacteria newly isolated from soil polluted with this contaminant. J Ferment Bioeng 86:539–544

Harayama S, Kok M, Neidle EL (1992) Functional and evolutionary relationships among diverse oxygenases. Annu Rev Microbiol 46:565–601

Hashimoto S, Fujita M (1987) Identification of three phenol-degrading microorganisms isolated from activated sludges and their characteristics. J Jpn Sewage Works 9:655–660

Hino H, Watanabe K, Takahashi N (1998) Phenol hydroxylase cloned from *Ralstonia eutropha* strain E2 exhibits novel kinetic properties. Microbiology 144:1765–1772

Hopkins GD, McCarty PL (1995) Field evaluation of in situ aerobic cometabolism of trichloroethylene and three dichloroethylene isomers using phenol and toluene as the primary substrates. Environ Sci Technol 29:1628–1637

Hopkins GD, Semprini L, McCarty PL (1993) Microcosm and in situ field studies of enhanced biotransformation of trichloroethylene by phenol-utilizing microorganisms. Appl Environ Microbiol 59:2277–2285

Hopper DJ, Chapman PJ, Dagley S. (1970) Metabolism of L-malate and D-malate by a species of *Pseudomonas*. J Bacteriol 104:1197–1202

Krumme ML, Timmis KN, Dwyer DF (1993) Degradation of trichloroethylene by *Pseudomonas cepacia* G4 and the constitutive mutant strain G4 5223 PR1 in aquifer microcosms. Appl Environ Microbiol 59:2746–2749

Mars AE, Prins GT, Wietzes P, Koning W, Janssen DB (1998) Effect of trichloroethylene on the competitive behavior of toluene-degrading bacteria. Appl Environ Microbiol 64: 208–215

McCarty PL, Golts MN, Hopkins GD, Dolan ME, Allan JP, Kawakami BT, Carrothers TJ (1998) Full-scale evaluation of in situ cometabolic degradation of trichloroethylene in groundwater through toluene injection. Environ Sci Technol 32: 88–100

Mu DY, Scow KM (1994) Effect of trichloroethylene (TCE) and toluene concentrations on TCE and toluene biodegradation and the population density of TCE and toluene degraders in soil. Appl Environ Microbiol 60:2661–2665

Semprini L (1997) Strategies for the aerobic co-metabolism of chlorinated solvents. Curr Opin Biotechnol 8:296–308

Semprini L, Roberts PV, Hopkins GD, McCarty PL (1990) A field evaluation of in-situ biodegradation of chlorinated ethenes: part 2, results of biostimulation and biotransformation. Ground Water 29:239–250

Semprini L, Hopkins GD, Roberts PV, Grbic-Galic D, McCarty PL (1991) A field evaluation of in-situ biodegradation of chlorinated ethenes: part 3, studies of competitive inhibition. Ground Water 29:239–250

Shih CC, Davey ME, Zhou J, Tiedje JM, Criddle CS (1996) Effects of phenol feeding pattern on microbial community structure and cometabolism of trichloroethylene. Appl Environ Microbiol 62:2953–2960

Speitel GE Jr, Thompson RC, Weissman D (1993) Biodegradation kinetics of *Methylosinus trichosporium* OB3b at low concentrations of chloroform in the presence and absence of enzyme competition by methane. Water Res 27:15–24

Watanabe K, Hino S, Onodera K, Kajie S, Takahashi N (1996) Diversity in kinetics of bacterial phenol-oxygenating activity. J Ferment Bioeng 81:560–563

Watanabe K, Teramoto M, Futamata H, Harayama S (1998) Molecular detection, isolation, and physiological characterization of functionally dominant phenol-degrading bacteria in activated sludge. Appl Environ Microbiol 64:4396–4402

Yamamoto S, Harayama S (1998) Phylogenetic relationships of *Pseudomonas putida* strains deduced from the nucleotide sequences of *gyrB*, *rpoD* and 16 S rRNA genes. Int J Syst Bacteriol 48:813–819

Yang RD, Humphrey AE (1975) Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. Biotechnol Bioeng 17:1211–1235