APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1997, p. 4145–4149 Vol. 63, No. 11
0099-2240/97/$04.00+0

Monitoring of an Alkaline 2,4,6-Trichlorophenol-Degrading Enrichment Culture by DNA Fingerprinting Methods and Isolation of the Responsible Organism, Haloalkaliphilic *Nocardioides* sp. Strain M6

OLGA MALTSEVA* AND PATRICK ORIEL

Center for Microbial Ecology and Department of Microbiology, Michigan State University, East Lansing, Michigan 48824

Received 22 May 1997/Accepted 14 August 1997

A site situated near Alkali Lake (Oregon) and highly contaminated by chloroaromatic compounds was chosen for isolation of alkaliphilic chlorophenol-degrading bacteria. Prolonged cultivation of an enrichment culture followed by successive transfers resulted in a strong increase in the 2,4,6-trichlorophenol (2,4,6-TCP) degradation rate. Repetitive extragenic palindromic PCR and amplified ribosomal DNA restriction analysis were applied to distinguish members of the enrichment culture and monitor them during the enrichment procedure. Comparison of the fingerprints of the isolates obtained from the enrichment culture and its total DNA fingerprint indicated the presence of an unidentified bacterium in the enrichment culture, assisting in its isolation. The 2,4,6-TCP-degrading isolate, M6, was tentatively identified as a *Nocardioides* sp. strain based on its partial 16S RNA sequence and fatty acid profile. Strain M6 was capable of utilizing up to 1.6 g of 2,4,6-TCP per liter as a sole carbon and energy source and could also grow on 2,4-dichlorophenol and 2,4,5-trichlorophenol. A high-cell-density suspension of this strain degraded a wide range of chlorinated phenols from di- to pentachlorophenol while showing a clear preference for phenols containing chlorine substituents in positions 2 plus 4. Based on its optimal pH (9.0 to 9.4) and sodium ion concentration (0.2 to 0.4 M) for growth, *Nocardioides* sp. strain M6 is a slightly halophilic alkaliphile.

Extensive use of chlorinated phenols and their derivatives in agriculture and industry has resulted in extensive environmental contamination. For example, conventional chlorine bleaching of kraft pulp has been estimated to produce approximately 100 to 300 g of chlorinated phenolic compounds per ton of pulp (9, 20). 2,4,6-Trichlorophenol (2,4,6-TCP), 2,3,4,6-tetrachlorophenol, and pentachlorophenol were the principal wood preservatives for many years worldwide (17, 18). 2,4-Dichlorophenol (2,4-DCP) and the very persistent compound 2,4,5-trichlorophenol (2,4,5-TCP) are generated in the environment from the extensively used agricultural biocides 2,4-dichloro- and 2,4,5-trichlorophenoxyacetic acid (7). Chlorinated phenols have inherent toxicities to biological systems, resulting in their persistence and accumulation in the environment. 2,4,5-TCP, 2,4,6-TCP, and pentachlorophenol have been placed on the U.S. Environmental Protection Agency's list of priority pollutants (21).

Several strains of bacteria have been described for their ability to completely mineralize polychlorinated phenols (3). However, none of them is capable of chlorophenol degradation under conditions of high alkalinity. We have initiated a project to isolate and examine alkaliphilic bacteria capable of polychlorophenol degradation. Understanding chlorophenol degradation under alkaline conditions is of interest for a number of reasons. (i) It is known that sorption of chlorophenols to organic matter is pH dependent (sorption decreases as pH increases) (3); therefore, an increase in pH can affect the bioavailability of chlorophenols and improve the efficiency of bioremediation. (ii) Since the toxicity of chlorophenols is usually attributed to the acid (undissociated form), the toxicity of a chlorophenol decreases as the pH increases (11, 22). Thus, chlorophenols should be less toxic to microorganisms at elevated pH values, which may allow bacteria to utilize them at higher concentrations. (iii) Alkaliphilic chlorophenol-degrading bacteria may be useful for bioremediation of alkaline sites contaminated with chlorophenols (8). Therefore, alkaliphilic microorganisms should provide a good opportunity to investigate chlorophenol degradation at high pH values and to aid in the development of effective bioremediation procedures.

In this paper we report on the use of molecular fingerprinting methods to assist in the isolation of an alkaliphilic and slightly halophilic bacterium, *Nocardioides* sp. strain M6, capable of growth on 2,4,6-TCP, 2,4-DCP, and 2,4,5-TCP and degradation of a wide range of chlorophenols in high-density culture.

MATERIALS AND METHODS

Site description and sample collection. A site situated near Alkali and West Alkali Lake (Oregon) and described earlier (19) was chosen to collect soil samples for enrichment. The site was highly contaminated in 1976 by release of 6,000 tons of 2,4-dichlorophenoxyacetic acid (2,4-D) production waste. Groundwater in the disposal area is approximately 1 m below the surface, has a pH 9.5 to 10, and is contaminated with high levels of mixed chlorophenols, 2,4-D, and related chloroaromatic compounds (8). Samples used in this study were taken within about 0.5 m from the surface in four different locations across the contamination plume in May 1994.

Media. Alkaline mineral medium 1 (AMM1), pH 9.5, described earlier (15), and alkaline mineral medium 3 (AMM3), pH 9.4, which differs from AMM1 by having lower concentrations of sodium chloride and sodium carbonates (20 g of NaCl per liter, 5 g of $NaHCO_3$ per liter, and 5 g of Na_2CO_3 per liter), were used in this investigation. TCP agar contained (per liter of AMM3) 20 g of agar and 200 mg of 2,4,6-TCP. Rich agar was supplemented with 1 g of yeast extract per liter and 1 g of Casamino Acids per liter.

Enrichment. Four soil samples collected at the Alkali Lake site were mixed together, and 5-g subsamples were used to inoculate Erlenmeyer flasks (500 ml)

* Corresponding author. Mailing address: 540 PSSB, Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824. Phone: (517) 353-7858. Fax: (517) 353-5917. E-mail: 23142ovm@msu.edu.

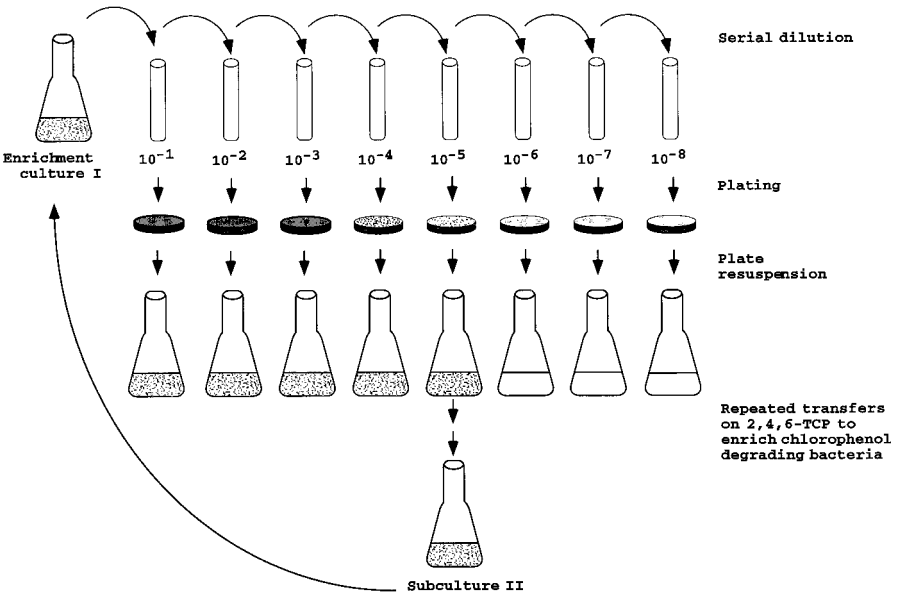

FIG. 1. Serial dilution-plate resuspension procedure, which was utilized to reduce Alkali Lake enrichment culture complexity. The whole procedure was sequentially repeated to obtain subcultures III and IV.

containing 200 ml of AMM1. Enrichment cultures were incubated at room temperature on a rotary shaker at 200 rpm with 2,4,6-TCP (20 mg/liter) as the sole carbon source. The concentration of the chlorophenol was monitored by high-performance liquid chromatography (HPLC). When the 2,4,6-TCP content decreased to 2 to 0 mg/liter, additional substrate (20 mg/liter) was added to the culture. After complete disappearance of 2,4,6-TCP, inoculum (10%, vol/vol) was transferred into fresh AMM3 supplemented with 40 mg of 2,4,6-TCP per liter. After several transfers of the enrichment culture, the initial concentration of chlorophenol was increased to 100 mg/liter and then to 200 to 500 mg/liter. The most active enrichment culture obtained was designated enrichment culture I.

Serial dilution of the enrichment cultures, molecular fingerprinting of their members, and isolation of a 2,4,6-TCP-degrading bacterium. A serial dilution technique was utilized to reduce the number of different phenotypes in the 2,4,6-TCP-degrading enrichment culture. For this, enrichment culture I was diluted 1:10 serially, and 0.05 ml of each dilution was plated onto TCP agar (Fig. 1). Plates were incubated for 10 to 15 days, and then each preparation was resuspended with 5 ml of AMM3, which was used to inoculate liquid AMM3 containing 50 mg of 2,4,6-TCP per liter and 20 mg of yeast extract per liter. A liquid culture from the highest dilution giving growth was transferred several times by using increased trichlorophenol (TCP) concentrations (from 100 to 500 mg/liter) and was designated subculture II. This complete procedure, which we call serial dilution-plate resuspension, was sequentially repeated to further reduce culture complexity (Fig. 1). DNA fingerprinting (repetitive extragenic palindromic [REP] PCR and amplified ribosomal DNA restriction analysis [ARDRA]) was used to differentiate and monitor isolated bacteria. The total DNA of the enrichment subcultures obtained in different steps of enrichment was also fingerprinted, and the patterns obtained were compared to the fingerprints of plated isolates. Isolates representative of each REP PCR pattern were tested for growth and degradation of 2,4,6-TCP in liquid AMM3.

Determination of optimal growth conditions. Cell growth was monitored by measuring culture optical density at 550 nm (OD_{550}). The salt dependence of growth was determined in AMM3 containing various concentrations of NaCl (0 to 2 M). The optimum pH for growth was determined by using 50 mM Tris-HCl buffer (pH 7.2 to 9.0) and 50 mM Na_2CO_3-$NaHCO_3$ buffer (pH 8.8 to 10.2). The media were supplemented with NaCl to keep the sodium ion concentration near 0.4 M.

Fatty acid analysis. Isolate M6 was grown in liquid AMM3 supplemented with 500 mg of 2,4,6-TCP per liter and 25 mg of yeast extract per liter for 3 days and then harvested by centrifugation, washed twice with 50 mM Tris-HCl buffer (pH 8.0), and stored at −20°C. A fatty acid methyl ether analysis of lipids was performed as described earlier (15).

Transformation of chlorinated phenols by cell suspensions. Isolate M6 was grown in AMM3 containing 500 mg of 2,4,6-TCP per liter and 25 mg of yeast extract per liter. At the end of the logarithmic growth phase cells were harvested by centrifugation, washed twice with fresh AMM3, and resuspended in the same medium to an OD_{550} of 3.0. Chlorinated phenols were supplied at concentrations 25, 50, and 100 mg/liter. The suspensions were shaken at 30°C, and samples were withdrawn after 0, 3, 6, and 24 h of incubation for chlorophenol analysis.

Quantitative determination of chlorophenols. Concentrations of chlorophenols in growth media were determined by HPLC as described earlier (15) by using 800 ml of methanol containing 0.2 ml of 85% *ortho*-phosphoric acid per liter as the mobile phase.

Extraction of DNA, PCR amplification, restriction analysis, and sequencing of PCR products. Enrichment subcultures II, III, and IV were grown on 2,4,6-TCP (500 mg/liter) for 48 to 56 h. Total genomic DNA of these subcultures was extracted by the miniprep method of Ausubel et al. (2) and used as a PCR template. Biomass from a single colony was used as the template for amplification of 16S RNA and REP sequences of the isolated bacteria. REP PCR was performed as described by deBruijn (4). The 16S rRNA genes were amplified and sequenced as described earlier (15).

RESULTS

Degradation of 2,4,6-TCP by enrichment cultures. The Alkali Lake enrichment cultures exhibited degradation of 2,4,6-TCP (20 mg/liter) after 2 months of cultivation, and after 3 additional months the chlorophenol was completely utilized. After prolonged cultivation followed by successive transfers, significantly increased rates of 2,4,6-TCP degradation were observed. Enrichment culture I, obtained after 1 year of enrichment, degraded 500 mg 2,4,6-TCP per liter in 7 to 8 days and showed no improvement with further transfers. Several attempts were made to isolate a pure TCP-degrading isolate by direct plating. More than 100 isolates obtained during different steps of the enrichment procedure were tested in liquid medium for growth and degradation of chlorophenol, but none of them proved capable of 2,4,6-TCP degradation.

Serial dilution, molecular fingerprinting of members of the enrichment culture, and isolation of the 2,4,6-TCP-degrading bacterium. Although single colonies capable of 2,4,6-TCP degradation were not isolated, a 2,4,6-TCP-degrading liquid culture was obtained by resuspending plated enrichment culture I. This suggested that either a TCP-degrading bacterium grew on the plates and remained unidentified or 2,4,6-TCP degradation required a consortium. The strategy which was chosen for further enrichment was to reduce the culture complexity by

serial dilution and utilize molecular fingerprinting to distinguish and monitor culture members. Transfers performed with the initial enrichment culture also indicated that decreased inoculum size drastically prolonged the lag time which is characteristic of growth on toxic substrates (1). Therefore, to increase the inoculum size, the serially diluted culture was plated, and the biomass obtained after 10 to 15 days of plate growth was resuspended and used to inoculate liquid TCP medium (for details on the serial dilution-plate resuspension procedure see Materials and Methods and Fig. 1).

Enrichment culture I was serially diluted and plated, and the liquid culture obtained from the highest dilution (10^{-5}) giving growth on 2,4,6-TCP was transferred several times with gradual increases in the 2,4,6-TCP concentration and designated subculture II (Fig. 1). Eight strains with different REP PCR patterns were isolated from subculture II. On the basis of the 16S RNA sequences (approximately 300 to 330 nucleotides), three isolates were tentatively identified as bacteria belonging to the family *Halomonadaceae*, two isolates were identified as *Rhizobium* strains, one isolate was identified as a *Flexibacter* sp., and one isolate was identified as an *Agromyces* sp. (data not shown). None of these organisms proved to be capable of 2,4,6-TCP degradation separately or in combination. These results suggested that subculture II contained unidentified bacteria participating in 2,4,6-TCP degradation. The REP PCR pattern of the DNA of subculture II contained bands corresponding to those of the eight isolated bacteria, but the pattern was too complex to distinguish bands from unidentified bacteria (data not shown).

Subculture II was subjected to further cycles of the serial dilution-plate resuspension procedure. 2,4,6-TCP-degrading subcultures III and IV contained only five and three of the eight original isolates, respectively. The bacteria isolated from subculture IV were designated M3 (a member of the *Halomonadaceae* sp.), M4 (*Rhizobium* sp.), and M5 (*Rhizobium* sp.). Comparison of the REP PCR patterns of these three bacteria with the REP PCR pattern of the total DNA from subculture IV, from which they were isolated, revealed a unique band in the subculture fingerprint (Fig. 2), which did not correspond to the bands of the three isolates mentioned above. To detect the unknown member, subculture IV was plated, and extensive REP PCR fingerprinting was performed. Each colony which appeared on the 10^{-5} dilution agar plates after 3 to 10 days of incubation was marked and checked for its REP PCR pattern, but all of the patterns obtained corresponded to those of isolates M3, M4, and M5. However, after 20 days of incubation very small uniform colonies appeared on TCP agar between marked and previously checked colonies. These colonies produced identical REP PCR patterns which differed from the fingerprints of strains M3, M4, and M5. The new isolate was designated M6. Comparison of the REP PCR patterns of strains M3 through M6 with the REP PCR pattern of subculture IV showed that the bands of M6 dominated in the subculture IV fingerprint, but the bands of the other three isolates were also present (Fig. 2). To further verify that the four isolated bacteria were the only bacteria present in subculture IV, subculture IV DNA and the DNAs of strains M3 through M6 were fingerprinted by ARDRA. *Rhizobium* sp. strains M4 and M5 presented the same ARDRA fingerprints with all six enzymes used for restriction, while the member of the *Halomonadaceae* (strain M3) and isolate M6 could be differentiated by their restriction patterns from each other and from the *Rhizobium* strains (Fig. 3 [data for three restriction enzymes are shown]). All of the ARDRA bands of subculture IV corresponded to bands of the four isolated bacteria, which confirmed that these bacteria were the only members of that liquid culture.

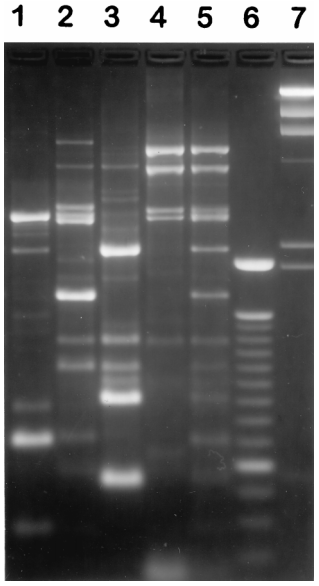

FIG. 2. REP-PCR patterns of Alkali Lake enrichment subculture IV grown on 2,4,6-TCP and four isolates obtained from this culture. Lane 1, strain M3 (a member of the *Halomonadaceae*); lane 2, *Rhizobium* sp. strain M4; lane 3, *Rhizobium* sp. strain M5; lane 4, *Nocardioides* sp. strain M6; lane 5, enrichment subculture IV; lane 6, 100-bp marker (bands from bottom to top: 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, and 2.075 kb); lane 7, λ *Hin*dIII marker (bands from bottom to top: 2.0, 2.3, 4.4, 6.6, 9.4, and 23.1 kb).

Strain M6 alone and strain M6 in combination with M3, M4, and M5 were checked for the ability to degrade 2,4,6-TCP. It was found that isolate M6 was capable of growth on and complete degradation of 2,4,6-TCP.

Characterization of the 2,4,6-TCP-degrading isolate. Colonies of M6 appeared on TCP agar within 18 to 20 days at 30°C

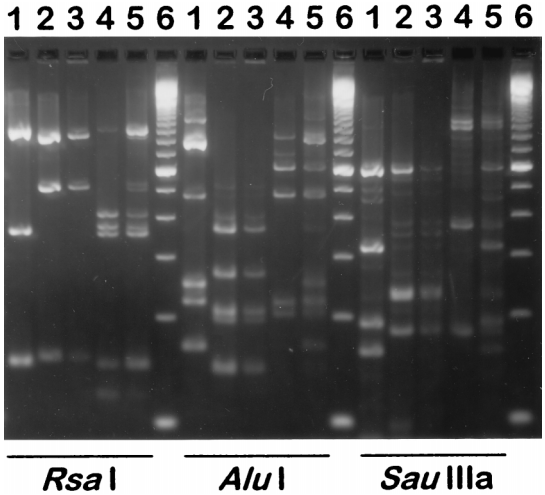

FIG. 3. ARDRA patterns of Alkali Lake enrichment subculture IV grown on 2,4,6-TCP and four isolates obtained from this culture. Lanes 1, strain M3 (a member of the *Halomonadaceae*); lanes 2, *Rhizobium* sp. strain M4; lanes 3, *Rhizobium* sp. strain M5; lanes 4, *Nocardioides* sp. strain M6; lanes 5, enrichment subculture IV; lanes 6, 100-bp marker (bands from bottom to top: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.2 kb). The restriction enzymes used were *Rsa*I, *Alu*I, and *Sau*IIIa.

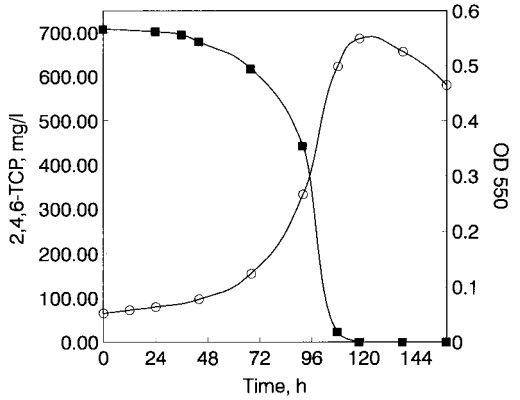

FIG. 4. Disappearance of 2,4,6-TCP (■) and increase in OD_{550} (○) during growth of isolate M6 on AMM3 supplemented with 2,4,6-TCP.

and reached a diameter of 0.5 to 1 mm in 2 weeks. No growth was detected on rich AMM3 agar. On the basis of a partial 16S RNA sequence (approximately 550 nucleotides), this strain was tentatively identified as a *Nocardioides* sp. The major fatty acids of isolate M6 (and their proportion ranges) were 16:0 iso (5.61 to 6.11%), 16:0 (7.88 to 8.31%), 17:1 *cis* 8 (6.48 to 7.05%), 17:0 (8.44 to 8.62%) and 18:1 *cis* 9 (37.26 to 37.35%).

Degradation of chlorophenols by a high-density cell suspension of *Nocardioides* sp. strain M6. Strain M6 was capable of utilizing 2,4,6-TCP as a sole carbon and energy source (Fig. 4). It could grow on 2,4,6-TCP at concentrations up to 1,600 mg/liter; however, the length of lag phase increased with increasing TCP concentration. This was especially vivid at 2,4,6-TCP concentrations higher than 400 to 500 mg/liter, where the lag phases were 24, 72, 144, and 288 h long at TCP concentrations of 400, 700, 1,200, and 1,600 mg/liter, respectively. *Nocardioides* sp. strain M6 was also capable of growth on 2,4-DCP (50 mg/liter) and 2,4,5-TCP (50 mg/liter). Other chlorophenols did not support growth.

Degradation of chlorophenols by a high-density cell suspension of *Nocardioides* sp. strain M6. A high-cell-density suspension of *Nocardioides* M6 completely degraded 2,4-DCP, 2,3,4-trichlorophenol, 2,4,5-TCP, or 2,4,6-TCP at a concentration of 100 mg/liter, 2,3,4,6-tetrachlorophenol at a concentration of 50 mg/liter, and 3,4-dichloro-, 2,3,4,5-tetrachloro-, or pentachlorophenol at a concentration of 25 mg/liter (Table 1). No degradation of monochlorinated phenol was found (Table 1). The strain showed a clear preference for phenolic substrates containing chloro substituents in positions 2 plus 4 (i.e., 2,4-DCP, 2,3,4-trichlorophenol, 2,4,5-TCP, 2,4,6-TCP, 2,3,4,5-tetrachlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol). The only exception to this was 3,4-dichlorophenol, which was also easily degraded by the resting cells. The dynamics of degradation of various chlorinated phenols were very similar, with the highest degradation rates observed during the first 1 h of incubation.

Salt and pH effects on the growth of *Nocardioides* sp. strain M6 on 2,4,6-TCP. *Nocardioides* sp. strain M6 grew on AMM3 supplemented with 500 mg of 2,4,6-TCP per liter and 20 mg of yeast extract per liter over a wide range of sodium ion concentrations (0 to 1.35 M), with the optimal concentration ranging from 0.2 to 0.4 M (Fig. 5a). The optimal pH for growth of this strain on medium containing 2,4,6-TCP (500 mg/liter) and 20 mg of yeast extract per liter was between 9.0 and 9.4 (Fig. 5b).

TABLE 1. Degradation of chlorinated phenols by resting cells of *Nocardioides* sp. strain M6[a]

Compound	% Degradation at an initial concn of:		
	25 mg/liter	50 mg/liter	100 mg/liter
2-Chlorophenol	0	0	0
3-Chlorophenol	0	0	0
4-Chlorophenol	0	0	0
2,3-Dichlorophenol	0	0	0
2,4-Dichlorophenol	100	100	100
2,5-Dichlorophenol	0	0	0
2,6-Dichlorophenol	0	0	0
3,4-Dichlorophenol	100	89	50
3,5-Dichlorophenol	0	0	0
2,3,4-Trichlorophenol	100	100	100
2,3,5-Trichlorophenol	0	0	0
2,4,5-Trichlorophenol	100	100	100
2,4,6-Trichlorophenol	100	100	100
2,3,4,5-Tetrachlorophenol	100	85	44
2,3,4,6-Tetrachlorophenol	100	100	72
2,3,5,6-Tetrachlorophenol	52	20	8
Pentachlorophenol	100	43	21

[a] Degradation was determined by HPLC (see Materials and Methods) after 24 h of incubation.

DISCUSSION

The usual approach for isolation of xenobiotic compound-degrading bacteria is direct plating of active enrichment cultures on minimal media with the toxicant as the only carbon source, followed by examination of the isolates for their degradative ability. Initial efforts to identify a 2,4,6-TCP-degrading bacterium from the Alkali Lake enrichment culture by this approach were unsuccessful. The main difficulty with this method is that microorganism growth on a toxic substrate is often slow and produces colonies with poorly defined morphology. Even on rich media it is frequently difficult to use morphology to distinguish bacteria belonging to the same taxonomic group selected by enrichment culture conditions. Molecular fingerprinting was therefore employed to differentiate and monitor bacteria obtained from the Alkali Lake enrichment culture. REP PCR, which can resolve very closely related isolates (4), and ARDRA, which can place isolates into different taxonomic groups (24), were both utilized for cultured isolate characterization.

When REP PCR was used, eight different phenotypes were identified among the plated bacteria in Alkali Lake enrichment subculture II. As expected, ARDRA was less sensitive and could not resolve two strains belonging to the *Halomonadaceae* (data not shown) and two *Rhizobium* strains (Fig. 3) which

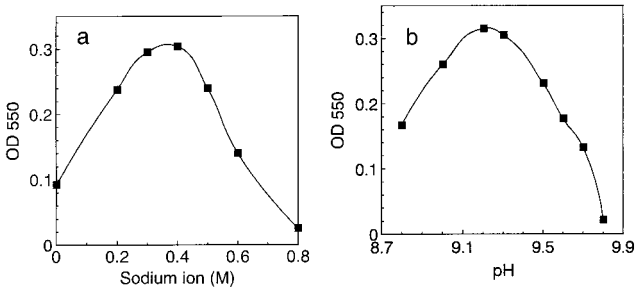

FIG. 5. Influence of sodium ions (a) and pH (b) on growth of isolate M6 on 2,4,6-TCP. The sodium ion concentrations of AMM3 were adjusted with NaCl; the pH values of AMM3 were adjusted with 50 mM Na_2CO_3-$NaHCO_3$.

were easily differentiated by REP PCR (Fig. 2). REP PCR also proved to be effective for monitoring bacteria during enrichment and isolation. In addition, REP PCR is fast and easy to use, since colony biomass can be directly used as a template for the PCR, eliminating the need for DNA isolation.

Fingerprinting of the total DNA of the enrichment culture and comparison with fingerprints of the isolates obtained from it were also useful because they indicated the presence of unidentified bacteria in the enrichment culture. Both REP PCR and ARDRA fingerprints of the total DNA of enrichment subculture IV contained unique bands from the 2,4,6-TCP-degrading strain, *Nocardioides* sp. strain M6, spurring attempts to isolate it. If isolation of an unknown strain present in an enrichment culture is difficult or impossible, the unique REP PCR bands can be used to make strain-specific probes (16), while unique 16S RNA bands can be utilized for determination of the taxonomic position of the unidentified bacterium. In this regard, it has been shown recently that phylogenetic information on bacteria can be helpful for developing strategies for their isolation from coculture or enrichment culture (10, 23).

The chlorophenol-degrading organism *Nocardioides* sp. strain M6 was well-adapted to the alkaline conditions at the Alkali Lake site, growing optimally at pH 9.0 to 9.4. Based on its salt and pH optima for growth, strain M6 should be regarded as a slightly halophilic alkaliphilic bacterium (6, 13). This strain is the first described extremophile which can grow on polychlorophenols and is the only known bacterium capable of utilization of both 2,4,6-TCP and 2,4,5-TCP. A high-density cell suspension of this strain can also degrade a wide range of chlorophenols from di- to pentachlorophenol. The ability of high-density cell suspensions to degrade a wider spectrum of chlorophenols has been previously reported for other 2,4,6-TCP-degrading strains (5, 12, 14). Compared with other 2,4,6-TCP-degrading bacteria, strain M6 has a unique spectrum of chlorophenol degradation. It is similar to *Pseudomonas pickettii* DTP0602 in showing a preference for substrates containing chloro substituents in positions 2 plus 4 but differs from the latter organism by exhibiting higher activity toward highly chlorinated phenols (from tri- to pentachlorophenols), while *P. pickettii* DTP0602 exhibits preferential activity for mono- and dichlorophenols.

An additional unique characteristic of *Nocardioides* sp. strain M6 is its ability to utilize 2,4,6-TCP at very high concentrations. This bacterium can grow on 2,4,6-TCP supplied as the only carbon source at a concentration of 1,600 mg/liter. Only one other strain, *Azotobacter* sp. strain GP1 (14), can utilize 2,4,6-TCP at concentrations near 1,000 mg/liter, and in contrast to strain M6, *Azotobacter* sp. strain GP1 has very narrow substrate specificity. Other known 2,4,6-TCP-degrading bacteria can grow on TCP only at concentrations below 200 to 400 mg/liter (5, 12). One of the possible explanations for the unique ability of M6 to utilize 2,4,6-TCP at very high concentrations is the decreased toxicity of chlorophenols at high pH values (3). This possibility will be examined in future investigations.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation Science and Technology Center grant BIR 9120006.

We thank Brian McClure of the Oregon Department of Environmental Quality for soil samples collected in 1994. We are grateful to Sandra Marsh for performing the fatty acid methyl ester analysis and to James Tiedje, Grace Matheson, John Dunbar, and Roberta Fulthorpe for helpful advice and stimulating discussions.

REFERENCES

1. **Andrews, J. F.** 1968. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. Biotechnol. Bioeng. **10:**707–723.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, and K. Struhl (ed.).** 1987. Current protocols in molecular biology, p. 2.4.1.–2.4.2. John Wiley & Sons, New York, N.Y.
3. **Bellin, C. A., G. A. O'Connor, and Y. Jin.** 1990. Sorption and degradation of pentachlorophenol in sludge-amended soils. J. Environ. Qual. **19:**603–608.
4. **deBruijn, F. J.** 1992. Use of repetitive sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. Appl. Environ. Microbiol. **58:**2180–2187.
5. **Golovleva, L. A., O. Zaborina, R. Pertsova, B. Baskunov, Y. Schurukhin, and S. Kuzmin.** 1992. Degradation of polychlorinated phenols by *Streptomyces rochei* 303. Biodegradation **2:**201–208.
6. **Grant, W. D., and B. J. Tindall.** 1986. The alkaline saline environment, p. 25–54. *In* R. A. Herbert and G. A. Cood (ed.), Microbes in extreme environments. Academic Press, London, United Kingdom.
7. **Häggblom, M. M., and R. J. Vallo.** 1995. Bioremediation of chlorophenol wastes, p. 389–434. *In* L. Y. Young and C. E. Cerniglia (ed.), Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss, Inc., New York, N.Y.
8. **Johnson, R. L., S. M. Brillante, L. M. Isabelle, J. E. Houck, and J. F. Pankow.** 1985. Migration of chlorophenolic compounds at the chemical waste disposal site at Alkali Lake, Oregon. 2. Contaminant distribution, transport, and retardation. Ground Water **23:**652–666.
9. **Jokela, J. K., and M. Salkinoja-Salonen.** 1992. Molecular weight distribution of organic halogens in bleached kraft pulp mill effluents. Environ. Sci. Technol. **26:**1190–1197.
10. **Kane, M. D., L. K. Poulsen, and D. A. Stahl.** 1993. Monitoring the enrichment and isolation of sulfate-reducing bacteria by using oligonucleotide hybridization probes designed from environmentally derived 16S rRNA sequences. Appl. Environ. Microbiol. **59:**682–686.
11. **Kishino, T., and K. Kobayashi.** 1995. Relation between toxicity and accumulation of chlorophenols at various pH, and their absorption mechanism in fish. Water Res. **29:**431–442.
12. **Kiyohara, H., T. Hatta, Y. Ogawa, T. Kakuda, H. Yokoyama, and N. Takizawa.** 1992. Isolation of *Pseudomonas pickettii* strains that degrade 2,4,6-trichlorophenol and their dechlorination of chlorophenols. Appl. Environ. Microbiol. **58:**1276–1283.
13. **Kushner, D. J., and M. Kamekura.** 1988. Physiology of halophilic bacteria, p. 109–138. *In* F. Rodriguez-Valera (ed.), Halophilic bacteria, vol. 1. CRC Press, Inc., Boca Raton, Fla.
14. **Li, D.-Y., J. Eberspächer, B. Wagner, J. Kuntzer, and F. Lingens.** 1991. Degradation of 2,4,6-trichlorophenol by *Azotobacter* sp. strain GP1. Appl. Environ. Microbiol. **57:**1920–1928.
15. **Maltseva, O., C. McGowan, R. Fulthorpe, and P. Oriel.** 1996. Degradation of 2,4-dichlorophenoxyacetic acid by haloalkaliphilic bacteria. Microbiology **142:**1115–1122.
16. **Matheson, V. G., J. Munakata-Marr, G. D. Hopkins, P. L. McCarty, J. M. Tiedje, and L. J. Forney.** 1997. A novel means to develop strain-specific DNA probes for detecting bacteria in the environment. Appl. Environ. Microbiol. **63:**2863–2869.
17. **McAllister, K. A., H. Lee, and J. T. Trevors.** 1996. Microbial degradation of pentachlorophenol. Biodegradation **7:**1–40.
18. **Nakamura, Y.** 1986. The present and future uses of antifungal wood preservatives. J. Antibacter. Antifung. Agents **14:**523–535. (In Japanese.)
19. **Pankov, J. F., R. L. Johnson, J. E. Houck, S. M. Brillante, and W. J. Bryan.** 1984. Migration of chlorophenolic compounds at the chemical waste disposal site at Alkali Lake, Oregon. I. Site description and ground-water flow. Ground Water **22:**593–601.
20. **Salkinoja-Salonen, M., M.-L. Saxelin, J. Pere, T. Jaakkola, J. Saarikoski, R. Hakulinen, and O. Koistinen.** 1981. Analysis of toxicity and biodegradability of organochlorine compounds released into the environment in bleaching effluents of kraft pulping, p. 1131–1164. *In* L. H. Keith (ed.), Advances in the identification and analysis of organic pollutants in water, vol. 2. Ann Arbor Science, Ann Arbor, Mich.
21. **Sittig, M.** 1981. Handbook of toxic and hazardous chemicals. Noyes Publications, Park Ridge, N.J.
22. **Stanlake, G. J., and R. K. Finn.** 1982. Isolation and characterization of a pentachlorophenol-degrading bacterium. Appl. Environ. Microbiol. **44:**1421–1427.
23. **Teske, A., P. Sigalevich, Y. Cohen, and G. Muyzer.** 1996. Molecular identification of bacteria from a coculture by denaturing gradient gel electrophoresis of 16S ribosomal DNA fragments as a tool for isolation in pure cultures. Appl. Environ. Microbiol. **62:**4210–4215.
24. **Vaneechoutte, M., R. Rossau, P. DeVos, M. Gillis, D. Janssens, N. Paepe, A. DeRourk, T. Fiers, G. Claeys, and K. Kersters.** 1992. Rapid identification of bacteria of the *Comamonadaceae* with amplified ribosomal DNA-restriction analysis (ARDRA). FEMS Microbiol. Lett. **93:**227–234.