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Isolation and Characterization of *Flavobacterium* Strains That Degrade Pentachlorophenol

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Bacteria able to mineralize 100 to 200 ppm of pentachlorophenol (PCP) were isolated by selective enrichment from PCP-contaminated soils from three geographic areas of Minnesota. Although differing somewhat in their responses to various biochemical and biophysical tests, all strains were assigned to the genus *Flavobacterium*. Five representative strains were examined in detail. All strains metabolized PCP as a sole source of carbon and energy; 73 to 83% of all carbon in the form of [U-^{14}C]PCP was returned as $^{14}CO_2$, with full liberation of chlorine as chloride. A comparison between strains in their ability to metabolize PCP showed some strains to be more efficient than others. Guanine-plus-cytosine contents of DNA ranged from 58.8 to 63.8%, and DNA/DNA hybridization studies with total DNA digests suggested substantial genetic homology between strains. All strains were shown to possess an 80- to 100-kilobase plasmid, and evidence suggested the presence of a larger plasmid (>200 kilobases).

Pentachlorophenol (PCP) and its salt (Na-PCP) are among the most widely distributed and versatile biocides used in the United States. PCP is lethal to a wide variety of organisms, both plant and animal, as an inhibitor of oxidative phosphorylation. Of all PCP produced, 80% is used by the wood preserving industry as a pesticide (fungicide, insecticide, and termiticide). The U.S. Environmental Protection Agency has registered an additional 578 products containing PCP, including products for use as herbicides, algicides, molluscicides, and disinfectants (13).

Much has been written about the effects of low-level PCP contamination on organisms such as fish, crabs, shrimp, oysters, clams, barnacles, and rats (2, 23, 24, 31). Evidence presented by Lu et al. suggests definite accumulation of PCP through the food chain (18). A number of studies conclude that contamination of tissues of human populations with PCP at a level of 10 to 20 ppb is average for industrialized societies (7, 17). PCP is thought to be mutagenic or at least comutagenic, and human exposure to PCP poses significant health hazards (7).

In cases of environmental contamination by PCP, traditional clean-up methods have included such techniques as the use of activated charcoal to decontaminate water. More recently, the industrial community has considered biological solutions to their problems with PCP. As is typical of chlorinated pesticides, PCP has proven to be resistant to biodegradation. However, the ability to degrade the biocide has been demonstrated among bacterial populations and fungi in both pure and mixed cultures (4, 6, 8, 15, 22, 28, 30, 33). Work by Edgehill and Finn (8) and Pignatello et al. (22) suggests the use of microbes as an alternative to the more traditional means of PCP removal from contaminated soils and water.

As far as we can determine, the literature makes no mention of a *Flavobacterium* sp. which possesses the ability to degrade PCP. However, there have been numerous reports of *Flavobacterium* sp. degrading a variety of chlorinated compounds and herbicides. Steenson and Walker (29) described the dissimilation of 2,4-dichlorophenoxyacetic acid through 2,4-dichlorophenol and 4-chlorocatechol, and MPCA (4-chloro-2-methylphenoxyacetic acid) through 4-chloro-2-methylphenol by *Flavobacterium peregrinum*. Bollag et al. (1) confirmed that *F. peregrinum* degraded MPCA to 4-chloro-2-methylphenol with full release of chlorine as chloride and conversion of the carboxyl carbon to volatile products. Burger et al. (3) isolated a *Flavobacterium* sp. that metabolized phenoxybutyric acids having chlorine on the aromatic ring; the organic chlorine was liberated, and the aromatic ring was cleaved. MacRae et al. (19) isolated a *Flavobacterium* sp. which degraded the pesticide 4-(2,4-dichlorophenoxy)butyric acid.

The herbicide asulam and the antibacterial agent sulphanilamide were degraded by a *Flavobacterium* sp. isolated by Walker (32). A *Flavobacterium* sp. isolated from paddy water by Sethunathan and Yoshida (25) decomposed diazinon to 2-isopropyl-6-methyl-4-hydroxypyrimidine which was then converted to CO_2. This bacterium also converted parathion to *p*-nitrophenol. Lastly, a *Flavobacterium* sp. degraded isopropyl-*n*-3-chlorophenylcarbamate to 3-chloroaniline and chloride ions in the soil perfusion systems of Kaufman and Kearney (14). This isolate was also found to degrade 2-chloroethyl-*n*-3-chlorophenylcarbamate, but at a much slower rate.

Work presented here introduces bacteria of the genus *Flavobacterium* isolated from PCP-contaminated soil which use PCP as their sole source of carbon and energy.

MATERIALS AND METHODS

Soil samples for enrichment cultures. Soil samples were collected from five geographic areas in Minnesota ranging in distance between 4 and 185 miles of each other. Each site possessed a history of PCP contamination. The sites were designated A, B, C, J, and M (see Table 1). Samples were retrieved from 30 cm below the ground surface at sites A, B, C, and J. At site M, a man-made stream, samples were taken from sediments that had been supplied with PCP-dosed river water for a period of several weeks (22).

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TABLE 1. Characteristics of soil sampling sites

Site	μg of PCP/g of soil (dry wt)	History of PCP contamination	Composition of soil[a]	Level of contamination with compounds other than PCP[b]	Moisture content
A	181	3 weeks	1, 2	Low, A	Very low
B	873	>10 years	1, 2, 3, 4	Moderate, B	Moderate
C	235	>10 years	1, 2, 3, 4	Moderate, C	Moderate
J	5,609	>10 years	1, 2, 3	Very high, C	Moderate-saturated
M	12	3 months	1, 2, 3	Negligible	Saturated

[a] 1, Sand; 2, gravel; 3, organic soil; 4, woodchips plus soil.
[b] A, bis(tributyltin)oxide; B, metals and creosote; C, metals, creosote, many polynuclear aromatic compounds

Enrichment procedures. All microbial isolation procedures were performed in media with a mineral salts (MS) base of the following composition (in grams of ingredient per liter): $NaNO_3$, 0.5; K_2HPO_4, 0.65; KH_2PO_4, 0.17; and $MgSO_4$, 0.1. Pentachlorophenol (+99% PCP; Sigma Chemical Co., St. Louis, Mo.) served as the sole carbon source. PCP was added to the MS base in the form of sodium pentachlorophenate. PCP was added directly to the MS base before being autoclaved (designated as PCP medium) and the final pH was ca. 7.3. Wet unsieved soil (5 g) from each site was used to inoculate separate 100-ml flasks of PCP medium containing 40 μg of PCP per ml. The cultures were placed on a shaker (200 rpm) at 30°C overnight. This step served two purposes: to remove bacteria from particle surfaces and to induce PCP-degradative enzymes. Cultures were centrifuged at 2,000 rpm for 10 min to remove particulate matter, and 1 ml of each supernatant solution served to inoculate the same medium contained in simple, replacement medium batch cultures. An uninoculated flask served as a control. The batch cultures consisted of six 1-liter flasks each containing 700 ml of PCP medium (40 μg of PCP per ml). Sterile, humidified air was bubbled through the medium at a rate of 5 ml/min as the sole means of agitation. Each flask was vented through a sterile filter and incubated at 25°C. Sterile glass wool (3 cm^2) was added to the flasks to facilitate attachment of bacteria. The biodegradation of PCP in each chemostat was monitored daily with two methods, measuring the adsorbance of the culture fluid at 318 nm (the λmaximum of PCP) and quantitating chloride evolution. Small samples from each flask were centrifuged to remove microbial cells and tested for $A_{318-320}$ versus a distilled water blank in 1-cm cells with a Beckman Model 25 spectrophotometer. Studies not described here indicated less than 2% adsorption of PCP to microbial cells. Free chloride ion concentrations in chemostat samples were measured by the method described by Pignatello et al. (22) with a Cl^- specific electrode. All measurements were compared to the uninoculated control to ensure that any degradation observed had been achieved through biological means. When degradation of PCP in flasks was nearly complete, spent medium and cells were carefully decanted from the flasks, leaving the glass wool. Fresh PCP medium (40 μg of PCP per ml) was then added. This was repeated until degradation of PCP at a level of 40 μg/ml was completed within 3 days, at which point the concentration of PCP in the medium was increased to 100 μg/ml and eventually to 200 μg/ml under the same conditions.

Loopfuls of medium from each flask were streaked onto plates containing low-buffer PCP medium (K_2HPO_4, 0.065 g/liter; KH_2PO_4, 0.017 g/liter; $MgSO_4 \cdot 7H_2O$, 0.1 g/liter; $NaNO_3$, 0.5 g/liter; PCP, 100 μg/ml), 1.0% purified agar and bromothymol blue (20 mg/liter) as a pH indicator. Plates were incubated at 30°C. Colonies which turned the agar yellow (HCl producers) were restreaked onto plates containing PCP medium (PCP at 100 μg/ml), 0.002% yeast extract (Difco Laboratories, Detroit, Mich.), and 1.0% purified agar. Plates were incubated at 30°C. Colonies that appeared were checked for purity. A dissecting microscope was used in examining colony morphology. Colonies were transferred three to five times between plates containing bromothymol blue and yeast extract before the ability to degrade PCP and apparent culture purity were consistent.

Each strain was rechecked for its ability to degrade PCP in liquid by inoculating standard test tubes (18 by 150 mm) containing 5 ml of low-buffer PCP medium and bromothymol blue. An uninoculated tube and one which was inoculated with *Escherichia coli* served as controls. The tubes were attached to a shaker (300 rpm) on a slant to provide for aeration and were incubated at 30°C. PCP degradation resulted in HCl release, and the medium turned yellow.

Examination of PCP mineralization abilities served to narrow the number of strains which would be used in further studies. Serum bottles (60 ml) were filled with 25 ml of PCP medium (PCP at 100 μg/ml) which had been spiked with [U-^{14}C]PCP (4 mCi · $mmol^{-1}$, >98% purity; California Bionuclear Corp., Sun Valley, Calif.) equaling 127,000 dpm per bottle and autoclaved. After cooling, each bottle received a 100-μl inoculum from a liquid bromothymol blue culture (see above). Serum bottles were capped with 20-mm Teflon-silicone Tuf-bond disks (Pierce Chemical Co., Rockford, Ill.), Teflon side down, and sealed with 20-mm aluminum seals (Pierce) to give an airtight fit. Incubation was at 30°C, stationary, for 2 weeks. Flushing of evolved $^{14}CO_2$ was performed at the end of 2 weeks. Compressed air was used to displace the $^{14}CO_2$ into an ethanolamine-supplemented scintillation counting fluid (22) for counting. Strains which returned at least 20% of the [U-^{14}C]PCP as $^{14}CO_2$ were retained for further characterization.

Characterization of PCP-degrading bacterial strains. A total of 40 selected strains were Gram stained. All were determined to be gram negative. This was confirmed by using the potassium hydroxide test of Gregersen (9). Each strain was tested for the presence of one or more plasmids by modification of the method of Crosa and Falkow (5); plasmids were separated by gel electrophoresis (0.7% gel, 18 h, 20 mA). Of the 40 strains, 11 were chosen for detailed study based on the following criteria: distinctive restriction patterns of DNA digested with endonuclease *Eco*RI, morphology, good growth at 30°C, and source of isolation. Extensive characterization of these strains (D. L. Saber, Ph.D. thesis, University of Minnesota, Navarre, in preparation) with the OXI-FERM TUBE system (Roche Diagnostics, Nutley,

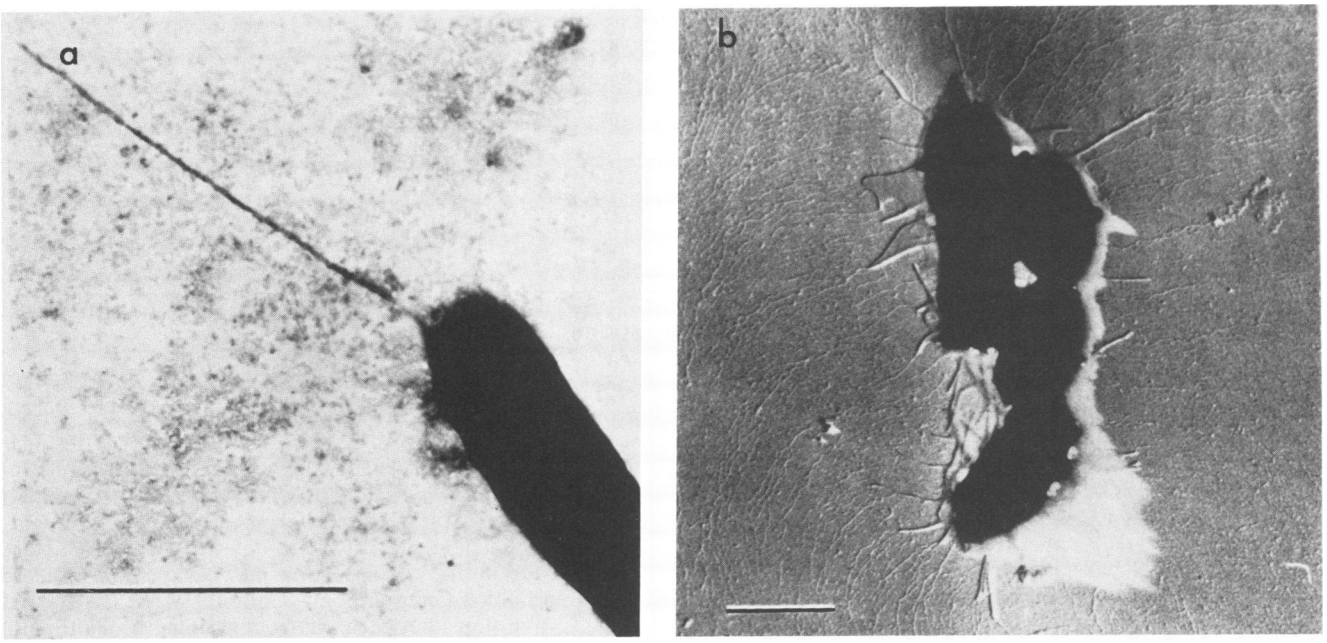

FIG. 1. (a) Electron micrograph of *Flavobacterium* sp. strain 8. Cells were stained with uranyl acetate. Straight, nonfunctional flagella present. (b) Electron micrograph of a group of *Flavobacterium* sp. strain 8. The thick polysaccharide which is produced by the cells causes clumping.

N.J.) and the methods of Holmes et al. (11), Weeks (34), Smibert and Krieg (26), Hendrie and Shewan (10), Kodata et al. (16), and Falkow (8a) indicated that they all belong in the genus *Flavobacterium* (see below). The guanine-plus-cytosine (G+C) content of six strains was obtained by using total DNA isolation procedures of Marmur (21) and the thermal melting profile method described by Johnson (12). Calculation of base composition from Tm was determined according to the equations of Mandel and Marmur (20), using *E. coli* b (mol% G+C = 51) as standard.

Comparison of strains. Five strains were compared for their efficiency in degrading PCP over time, using the equation: degradative parameter/micrograms/milliliter of protein/hour. The four parameters used were chloride evolution, concentration of PCP as measured by gas chromatography (22), decrease in pH (HCl release) and A_{318} of culture fluid. Three 2-liter flasks containing 1 liter of PCP medium (100 μg/ml) were inoculated with a 20-ml culture of the test strain which had previously been grown on the same medium to mid-logarithmic stage. Each flask was sealed with a rubber stopper penetrated by three glass tubes: one to serve as a vent, one through which air would be bubbled, and one for removal of samples. A similar fourth flask containing PCP medium (100 μg/ml) plus formaldehyde to a final concentration of 2% served as a killed control. The flasks were maintained on a shaker (200 rpm) at 30°C. Samples were removed at the start and approximately every 3 h postinoculation. Just before sampling, flasks were attached to a regulating manifold apparatus serviced by air which was prescrubbed of CO_2 and humidified by bubbling through 5 N NaOH–2 N NaOH–distilled water. Flasks were flushed for 1 h. Samples (35 ml) were then withdrawn from each flask for measurement of free chloride ion concentrations and monitoring of PCP concentration via spectrophotometer readings at 318 nm. pH measurements were taken using an Accumet pH meter (model 825 MP) with attached combination electrode with calomel reference. A two-point calibration was used; the instrument was recalibrated before measurement of each sample. PCP concentrations were monitored with a Hewlett Packard 5790A series gas chromatograph with a 3390A integrator by using the method of Pignatello et al. (22).

Total protein measurements were derived from 200-ml samples taken at the beginning, middle, and end of the experiment. Cells were centrifuged at 12,100 × *g* for 20 min, washed once with sterile MS buffer, and frozen. Cells were then thawed and lysed with 1 N NaOH. Protein measurements were made by the Coomassie brilliant blue method (Bio-Rad protein assay; Bio-Rad Laboratories, Richmond, Calif.) following the instructions of the manufacturer.

Strains were compared in their abilities to convert [U-^{14}C]PCP to $^{14}CO_2$. Three 250-ml flasks containing 100 ml of PCP medium were inoculated with growing cultures, as stated above; a fourth flask served as a killed control. Each flask received 500,000 dpm of [U-^{14}C]PCP. The flasks were sealed to airtightness by stoppers holding two glass tubes with attached rubber tubing and clips: one serving to bubble fresh, sterile air through the medium and one through which $^{14}CO_2$ could be flushed into scintillation-trapping fluid (22). These flasks were inoculated simultaneously with the larger flasks and inoculated from the same stock culture. They were incubated under similar conditions and flushed along with the larger flasks.

Five of the strains were tested for their degree of homology by using the DNA/DNA hybridization technique of Southern (27). The total DNA from these strains (isolated by the method of Marmur [21]) had been digested with the endonuclease *Sal*I (as per the manufacturer's recommendations). Bacteriophage lambda (*Sal*I digest) served as the control.

Electron microscopy. Cells were fixed in 2% glutaraldehyde for ~1 h, washed twice with 0.1 M cacodylate buffer, and suspended in similar buffer. One drop of cell

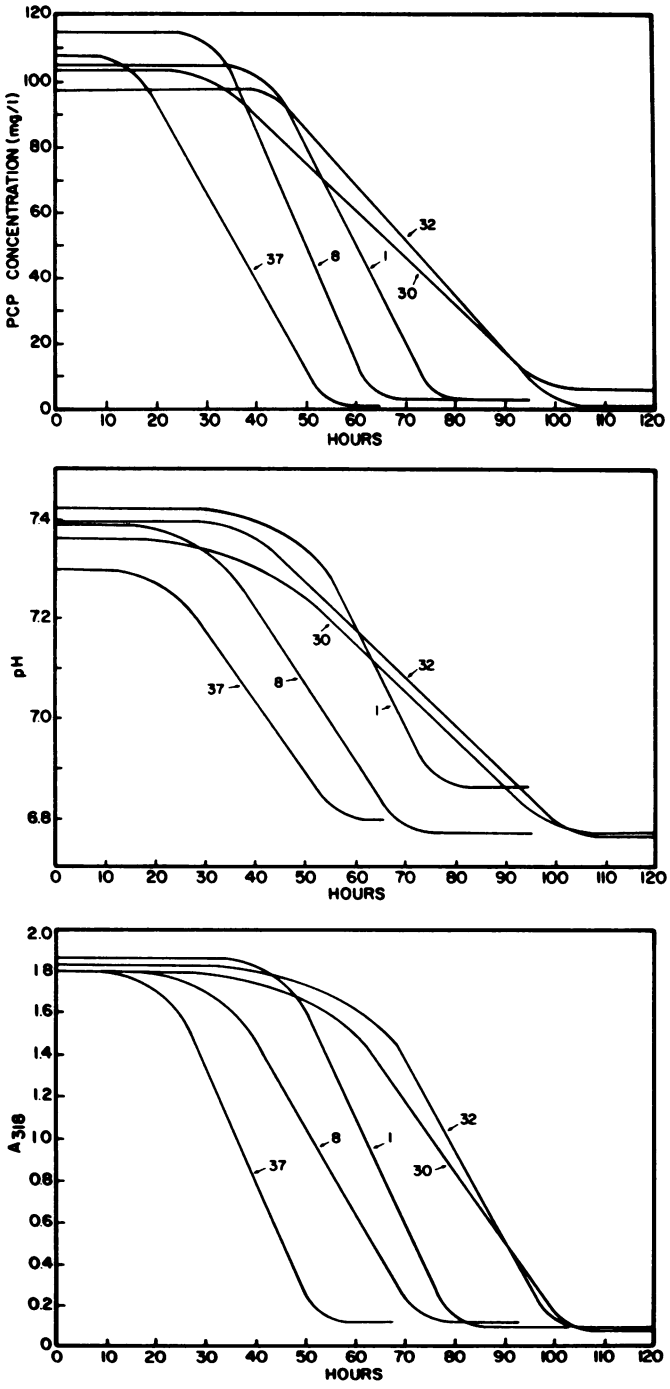

FIG. 2. Comparison of five *Flavobacterium* strains with the degradative parameters: PCP concentration (a), decrease in pH (b), and decrease in optical density at 318 nm (c). Numbers denote various strains.

suspension was placed on a glow-discharged, carbon-coated 200-mesh grid and allowed to stand for 1 min. Excess suspension was drawn off, and the grid was stained with 1% uranyl acetate for 1 min. Photographs were taken with a Hitachi 6010 STEM operating at 75 KeV. Shadowed preparations were fixed similarly, and then cells were placed in a Denton vacuum evaporator and rotary shadowed with platinum-palladium.

RESULTS

Isolation of PCP-degrading bacteria. Soil samples taken from five geographic sites in Minnesota yielded numerous closely related strains of bacteria capable of degrading PCP. Environmental concentrations of PCP at the sites varied between 12 and 5,609 μg/g of dry soil, and the history of PCP contamination ranged from 3 weeks to over 10 years, as summarized in Table 1. Enrichment cultures with a defined PCP medium (MS base with pure PCP, 40 μg/ml) contained in the flasks of simple replacement medium batch cultures were effective. PCP was degraded to a level below detection by spectrophotometric analysis within 2 to 3 weeks of initial inoculation of enrichments from three of the five sites as follows: sites B and M, 2 weeks; site C, 3 weeks. No PCP degradation was evident in the flasks inoculated from sites J and A after 5 weeks. Minimal bacterial growth appeared on nutrient agar plates streaked with soil suspensions from site A, and fungal growth only covered similar plates inoculated with soil suspensions from site J. Chloride ion concentrations in flasks B, M, and C increased from an initial average molarity of 3.05×10^{-4} to a final average molarity of 1.2×10^{-3}. Spent PCP medium was decanted and replaced with a fresh portion of identical medium. After 4 weeks of adaptation, degradation of 40 μg of PCP per ml occurred within 3 days in all active enrichments. The concentration of PCP in the enrichment medium was increased and maintained at 100 μg/ml for an additional 3 weeks until total degradation of this amount was achieved in 3 days. A final increase to 200 μg/ml was maintained through the remainder of the isolation procedure.

There was no apparent correlation between PCP degradation and the extent of bacterial growth in the flasks. Visible growth (turbidity) was negligible. Loopfuls of media from the flasks were used to inoculate plates containing PCP as the sole source of carbon. In attempting to isolate single colonies of PCP-degrading bacteria, it was evident that the majority of colonies which appeared on the PCP plates did not turn the indicator dye, bromothymol blue, to the characteristic yellow color associated with HCl release that accompanies degradation of PCP. Colonies which did exhibit a color change were often small and easily masked by larger spreading colonies. A dissecting microscope was used throughout the isolation procedure; the small PCP-degrading colonies were picked while examined through the microscope. Numerous transfers between plates of PCP medium plus bromothymol blue (to monitor for PCP degradation) and PCP medium plus yeast extract (to help ensure purity) were necessary. The final 85 strains believed to be pure cultures of PCP-degrading bacteria were tested for their ability to degrade PCP in liquid cultures containing PCP and bromothymol blue. All 85 of the strains proved to be positive for PCP degradation; 34 strains were derived from site B, 41 strains were from site M, and 10 strains were from site C. Throughout the isolation procedure, no degradation of PCP occurred in the flasks inoculated from sites J and A. These flasks were checked routinely for the presence of PCP-degrading bacteria, but none were isolated.

Using all 85 strains, those which returned the greatest percentage of $^{14}CO_2$ from [U-^{14}C]PCP were chosen for further characterization. Of the strains, 40 returned between 20 and 23% of the parent molecule as $^{14}CO_2$ in the initial serum bottle experiment.

Characterization of PCP-degrading bacteria. Different

TABLE 2. Comparison of degradative parameters of PCP-degrading *Flavobacterium* strains

Isolate	Decrease in pH (U)	Evolution of choride (molarity)	Decrease in OD[c] at 318 (U)	Decrease in PCP (μg/ml)	Avg efficiency
1	0.066[a] (100)[b]	1.1×10^{-4} (100)	0.162 (100)	9.35 (100)	100
37	0.046 (69.7)	7.0×10^{-5} (63.6)	0.11 (51.2)	7.11 (76)	65.1
8	0.03 (45.5)	5.55×10^{-5} (50.5)	0.078 (48.1)	6.49 (69.4)	53.4
30	0.018 (27.3)	3.9×10^{-5} (35.5)	0.066 (40.7)	2.7 (28.9)	33.1
32	0.009 (13.6)	7.9×10^{-6} (7.18)	0.055 (25.9)	1.58 (16.9)	15.9

[a] All rates are based on the equation: degradative parameter/micrograms/milliliter of protein/hour.
[b] The number in parentheses denotes efficiency of rates relative to other isolates in that category.
[c] OD, Optical density.

strains of bacteria were isolated from sites B, C, and M. The strains were differentiated from each other by extensive characterization with a wide variety of biochemical and biophysical tests (D. L. Saber, Ph.D. thesis). All strains were identified as being of the genus *Flavobacterium*. Of particular importance was the test for motility which distinctly separates these strains from the genus *Pseudomonas*. The cells were nonmotile in soft agar and in hanging drop preparations. No flagella were observed when cells were flagellum-stained and compared to a *Pseudomonas* sp. Further confirmation of nonflagellation required the use of an electron microscope. Electron micrographs of the various strains indicated a type of straight, polar appendage typically present in only 2% of the cells in any given field (Fig. 1a). These structures were considered to be nonfunctional flagella.

The cells produced a thick slime, presumedly a polysaccharide, particularly when grown on rich media (Fig. 1b). The cells were gram-negative, exhibiting a nondiffusible yellow pigment. They were 1.5 μm long and 0.4 μm wide and oxidase-, catalase-, and phosphatase-positive. The G+C content of the representative strains was between 58.8 and 63.8%; this is within the range known for *Flavobacterium* sp. derived from soil and water (11).

Comparison of five representative strains. The results of a comparison between the efficiencies with which five strains degraded PCP are summarized in Table 2. In assigning 100% to the most efficient degrader, values range downward to 15.9%. Figure 2 illustrates degradation of PCP as monitored by decrease in pH, spectrophotometer readings, and measured PCP concentrations. While the slope of the line generated by any one degradative parameter is important in determining the rate of degradation, the amount of protein produced by a strain may affect its overall efficiency. Using the equation: degradative parameter/micrograms/milliliter of protein/hour, strain 1 was most efficient, while strain 32 was the least efficient. All efficiencies of PCP degradation varied only within one order of magnitude (Table 2).

The strains were tested for their ability to degrade PCP completely to CO_2. Results summarized in Fig. 3 indicated that between 73 and 83% of all radiolabeled carbon in the form of [U-^{14}C]PCP was returned as $^{14}CO_2$. While this suggests that only 17 to 27% total carbon was asssimilated

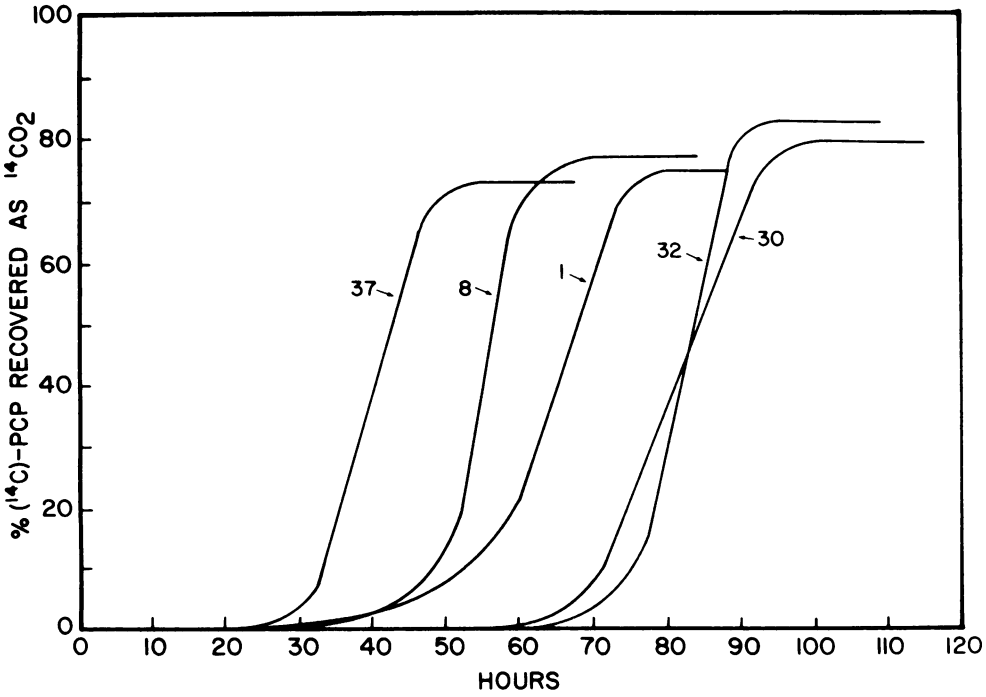

FIG. 3. Comparison of five *Flavobacterium* strains for their ability to metabolize ^{14}C-PCP to $^{14}CO_2$. Numbers denote various strains.

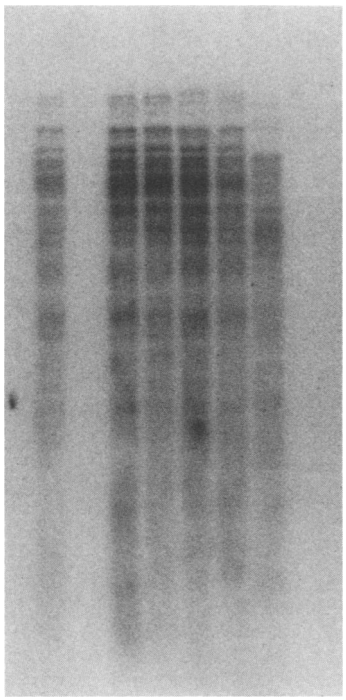

FIG. 4. Total DNA digests of six representative *Flavobacterium* strains. ^{32}P-DNA from strain 37 (left) was used to probe DNA digests of strains 1, L, 8, 30, and 32 (left to right); last lane, lambda DNA (no hybridization).

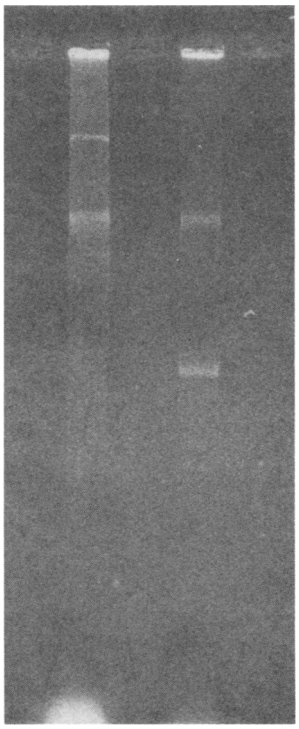

FIG. 5. Plasmids observed in *Flavobacterium* strain 32. Left lane, strain 32, uppermost band, suspected large (>200 kb) plasmid; lower two bands, two forms of an 80- to 100-kb plasmid. Right lane, Unrestricted plasmid pBR322 (4.3 kb).

into cell mass, it is consistent with the average maximum culture turbidity (A_{560}) of 0.038. Mineralization rates were very consistent, ranging between 3.7 and 7.2% of total [U-^{14}C]PCP returned as $^{14}CO_2$ per hour.

Genetic homology. Total DNA digests from five strains were compared with digested DNA from strain 37 by using DNA/DNA hybridization. Results indicate substantial homology between all strains (Fig. 4). Probe DNA from strain 37 hybridized extensively with DNA digests of all other PCP-degrading *Flavobacterium* strains. All strains contained similar plasmids, one 80- to 100-kilobase (kb) plasmid and probably a second of >200 kb (Fig. 5).

DISCUSSION

Several unique PCP-degrading *Flavobacterium* strains were isolated by enrichment culture from PCP-contaminated soils from three sites in Minnesota. Contaminated soils from 2 additional sites yielded no PCP-degrading microorganisms. These latter sites were either recently contaminated (3 weeks) with moderate amounts of PCP (181 μg/mg of dry soil, site A) or had been contaminated for an extended period with high (toxic) levels of both PCP (5,609 μg/mg of dry soil, site J) and other organic chemicals. Our failure to isolate PCP-degrading bacteria from sites A and J may be due to the following (Table 1): site A, soil composition (sandy), moisture content (very low) and length of exposure to PCP (only about 3 weeks, see reference 22); site J, very toxic levels of PCP and other organic contaminants.

All strains isolated were found to be members of the genus *Flavobacterium*. Eleven representative strains varied only in their abilities to utilize and ferment some carbon sources. Between 73 and 83% of all the carbon in [U-^{14}C]PCP was returned as $^{14}CO_2$ by five strains examined in detail. The saprophytic coryneform bacterium, KC-3, isolated by Kirsch and Etzel (15), returned 73% of the [U-^{14}C]PCP as $^{14}CO_2$ after 24 h (10 μg of PCP per h/mg of cell [dry weight]). While no direct, quantitative comparison may be made to the work by Kirsch and Etzel (no cell dry weights were measured here), it is clear that between 1.7 and 3.6 μg of PCP per ml was degraded per hour by all our strains, with an average of 0.65 μg/ml of total cell protein. This indicates that biodegradation abilities of our strains were roughly comparable to the ability of the organism discussed by Kirsch and Etzel.

It is possible that our isolation of only *Flavobacterium* species resulted from our particular enrichment protocol and defined medium. However, we used a defined medium similar to that employed by Watanabe (33), who isolated a PCP-degrading *Pseudomonas* strain. It is possible that the use of replacement medium batch cultures during the enrichment process favored isolation of *Flavobacterium* strains, although we have no explanation for why this might occur.

Of particular interest is the homology which exists between strains, as demonstrated by total DNA digests and DNA/DNA hybridizations. Since the strains were isolated from areas quite separated from one another (between 4 and 185 miles) the homology between strains in both DNA digests is noteworthy.

Each strain was shown to possess at least one plasmid (80 to 100 kb), and evidence suggested the presence of a second plasmid (>200 kb). Further studies of these plasmids will be reported at a later time.

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