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Numerical Taxonomy of Phenanthrene-Degrading Bacteria Isolated from the Chesapeake Bay

PAUL A. WEST,† GIDEON C. OKPOKWASILI, PHYLLIS R. BRAYTON, D. JAY GRIMES, AND RITA R. COLWELL*

Department of Microbiology, University of Maryland, College Park, Maryland 20742

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Phenanthrene-degrading bacteria were isolated from Chesapeake Bay samples by the use of a solid medium which had been overlaid with an ethanol solution of phenanthrene before inoculation. Eighteen representative strains of phenanthrene-degrading bacteria with 21 type and reference bacteria were examined for 123 characteristics representing physiological, biochemical, and nutritional properties. Relationships between strains were computed with several similarity coefficients. The phenogram constructed by unweighted-pair-group arithmetic average linkage and use of the simple Jaccard (S_J) coefficient was used to identify seven phena. Phenanthrene-degrading bacteria were identified as *Vibrio parahaemolyticus* and *Vibrio fluvialis* by their clustering with type and reference strains. Several phenanthrene-degrading bacteria resembled *Enterobacteriaceae* family members, although some *Vibrio*-like phenanthrene degraders could not be identified.

Hydrocarbon pollution of estuarine and marine environments occurs frequently. Removal of these pollutants by biodegradative processes has been a subject of extensive investigation (4), owing partly to their recalcitrance to biodegradation in the natural environment. Phenanthrene, a polycyclic aromatic hydrocarbon (PAH) that is present in coal tar and petroleum, is a by-product of coal coking, conversion of coal to synthetic fuel, and petroleum refining. Though not highly toxic (mouse oral 50% lethal dose, 700 mg/kg), phenanthrene and related PAHs are suspected carcinogens. Mussels (11) and fish (13) bioaccumulate PAHs, and there is evidence that PAHs are tumorigenic in marine animals (2, 3).

Strains of several genera, including *Pseudomonas* (1, 6), *Aeromonas* (10), *Vibrio* (8), and *Alcaligenes* (9), which have been isolated from soil, are capable of degrading PAHs, in particular phenanthrene. The application of recombinant gene technology for in vitro manipulation of such bacteria to produce cells possessing genes specific for the degradation of recalcitrant compounds is rapidly gaining importance. However, it is necessary to identify with confidence all strains before attempting genetic studies, manipulations, and ultimate application. We report here the identification by numerical taxonomic techniques of phenanthrene-degrading bacteria isolated from areas of the Chesapeake Bay subjected to hydrocarbon pollution. The degradative ability of these bacterial strains has been demonstrated with the use of [^{14}C]phenanthrene (G. C. Okpokwasili, C. C. Somerville, D. J. Grimes, and R. R. Colwell, manuscript in preparation). The strains may prove to be useful candidates for genetic engineering applied to biodegradative processes.

MATERIALS AND METHODS

Description of sampling sites. Baltimore Harbor is a well-known shipping terminal located on the western shore of the Chesapeake Bay. It is known to receive continuously an input of hydrocarbons, mainly as a result of tank-washing activities from ocean-going vessels, accidental spills, wastewater effluents, and nonpoint source runoff (20). Fort McHenry is located very close to Baltimore Harbor. In addition to the above sources of hydrocarbon contamination, this area receives dumping of waste coals from a nearby steel plant. Solomons Island is also located on the western shore of the Chesapeake Bay, approximately 100 km south of Baltimore, Md. The site of Solomons water collection was within 50 m of Solomons Harbor, which is used for recreational and small commercial fishing boats.

Isolation of phenanthrene-degrading bacteria. Samples of water and sediment were collected from the three Chesapeake Bay sites by using a sterile Niskin sampler (General Oceanics, Miami, Fla.) and a petite ponar grab (Wildlife Supply Co., Saginaw, Mich.), respectively. Bacteria capable of degrading phenanthrene were detected on estuarine salt water agar plates (14). The medium contained sodium glycerophosphate, 0.05 g; glycerol, 0.5 ml; proteose peptone, 0.1 g; yeast extract, 0.1 g; agar, 15 g; and aged Chesapeake water, 1 liter. After the plates were poured and dried overnight, 0.2 ml of a filtered (0.2 μm) ethanol solution (containing 0.5 g of phenanthrene per 100 ml of ethanol) was pipetted and uniformly spread on the surface of agar plates that were being spun on a turntable (16). The plates were dried overnight at 25°C to allow the ethanol to evaporate before inoculation. Plates prepared in this manner had an opaque, opalescent film of phenanthrene on the agar surface. Samples were inoculated within 15 min of collection. Inoculated plates were incubated at 25°C for 2 weeks. Phenanthrene-degrading (Phn^+) bacteria appeared as colonies surrounded by zones of clearing in the opaque phenanthrene, usually within 4 to 14 days. All Phn^+ bacteria were streaked for purity on estuarine salt water agar plates and stored at 25°C. The strains and their sources are listed in Table 1.

Reference strains. Type strains used are listed in Table 1. All strains were maintained on T1N1 + E agar containing tryptone (Difco Laboratories, Detroit, Mich.), 10 g; NaCl, 10 g; $MgCl_2 \cdot 6H_2O$, 4 g; KCl, 1 g; agar (Difco), 15 g; and deionized water, 1 liter.

Characterization tests. Unless otherwise stated, all media contained 1% (wt/vol) NaCl, 0.4% (wt/vol) $MgCl_2 \cdot 6H_2O$,

* Corresponding author.
† Present address: Ministry of Agriculture, Fisheries, and Food, Fisheries Laboratory, Burnham-on-Crouch, Essex CMO 8HA, England.

TABLE 1. Sources and designations of strains used

Strain	Designations[a]	Source
Phenanthrene degraders	GID7, GID9, GID10	
	GID12, GID16, GID17	Solomons (Md.) water
	GID18, GID19, GID20	
	GID21, GID22,	
	GID30, GID32, GID33, GID34	Baltimore Harbor (Md.) water
	GID53, GID54, GID55	Fort McHenry (Md.) sediment
Aeromonas hydrophila	ATCC 7966	Spoiled milk
	ATCC 15467	Oil emulsion
Vibrio alginolyticus	ATCC 17749	Spoiled mackerel
Vibrio anguillarum	ATCC 19264	Cod
Vibrio campbelli	ATCC 25920	Seawater
Vibrio cholerae	P688[b]	Sediment
	569-B	Human feces
	ATCC 14035	Human feces
Vibrio diazotrophicus	ATCC 33466	Sea urchin
Vibrio fischeri	ATCC 7744	Seawater
Vibrio harveyi	ATCC 14126	Amphipod
Vibrio fluvialis	NCTC 11327	Human feces
	NCTC 11328	River water
Vibrio natriegens	ATCC 14048	Marsh
Vibrio nereis	ATCC 25917	Seawater
Vibrio nigripulchritudo	ATCC 27043	Seawater
Vibrio parahaemolyticus	ATCC 17802	Seafood
Vibrio pelagius	ATCC 25916	Seawater
Vibrio splendidus	ATCC 25914	Seawater
	ATCC 33125	Marine fish
Vibrio vulnificus	ATCC 27562	Blood

[a] ATCC, American Type Culture Collection, Rockville, Md., NCTC, National Collection of Type Cultures, Colindale, London, England.
[b] See reference 19.

and 0.1% (wt/vol) KCl to satisfy the electrolyte requirement for growth. All characterization tests have been previously described (12, 18, 19) and were used without modification. The probability of an erroneous two-state character result was estimated by the procedure of Sneath and Johnson (17).

Computer analysis. All tests were coded as two-state binary characters and were scored 1 (positive) or 0 (negative) for the purpose of coding into the computer. Numerical taxonomic analyses were performed by use of the simple matching (S_{SM}) and Jaccard (S_J) types of similarity coefficient. Phenograms were constructed by single linkage and unweighted-pair-group arithmetic average linkage. Clustering procedures were made by using the TAXAN 6 program, and the feature frequencies of strains in each cluster were calculated by using program ICPS2. Both programs are available on the UNIVAC 1180 computer system available at the University of Maryland, College Park.

RESULTS

Data matrix. Nineteen characters (listed as follows), although useful for classification, had no differentiating value and were eliminated from the data matrix before computer analyses were performed. All strains were gram negative and were positive for growth in 3% NaCl and at pH 10.0; acid production from trehalose; growth on D-glucose as sole carbon source; and resistance to bile salts (0.5%, wt/vol), neutral red (0.002%, wt/vol), and Teepol 610 (0.4%, wt/vol). All strains were negative for production of H_2S; degradation of xanthine; acid production from *m*-inositol; and growth on butyrate, valerate, ethanolamine, adenine, xanthine, dulcitol, *p*-hydroxybenzoate, and phenylacetate as sole carbon sources. The final matrix was prepared by using combined data for 123 characters on 39 strains. The probability for test errors was estimated to be 5.4% by the method of Sneath and Johnson (17).

Description of phena. Similarities between strains were calculated and phenograms constructed by single linkage and average linkage (unweighted-pair-group arithmetic average). Similar groupings were obtained by both techniques. The phenogram constructed by unweighted-pair-group arithmetic average linkage with the S_J similarity coefficient is shown in Fig. 1. Identification of seven phena was made, and

the feature frequencies of all characteristics tested for each strain in the phena are listed in Table 2.

Phenon 1 formed at the 90.4% similarity level and was identified as *Vibrio parahaemolyticus*. The phenon contained three strains, GID9, GID12, and ATCC 17802. Typically, none fermented sucrose, grew at 10% NaCl, or gave a positive Voges-Proskauer reaction. All strains grew on ethanol, L-leucine, and putrescine.

Phenon 2 was identified as *Vibrio fluvialis* and formed at the 79.6% similarity level. Four phenanthrene degraders, strains GID17, GID19, GID20, and GID21, were clustered with the type strain *V. fluvialis* NCTC 11327 and the recommended working strain NCTC 11328. All strains possessed properties described for *V. fluvialis* (12).

Phenon 3 did not contain any phenanthrene-degrading bacteria. It consisted of three type and reference strains of *V. cholerae*.

Phenon 4 formed at the 86.6% similarity level and contained two phenanthrene-degrading isolates, strains GID15 and GID18. Both strains were placed in the genus *Vibrio* by their sensitivity to 0/129, fermentative metabolism, and positive oxidase reaction. These strains most closely resembled *Vibrio vulnificus* but failed to show clustering with the type strain, and for this reason, phenon 4 strains remained unidentified.

Phenon 5, identified as *Aeromonas hydrophila*, formed at the 75% similarity level. It contained no phenanthrene degraders.

Phenon 6 formed at the 84.1% similarity level and contained four phenanthrene degraders, strains GID32, GID33, GID53, and GID54. All strains had been provisionally assigned to the genus *Vibrio* owing to their sensitivity to 0/129. However, the distinct clustering of this phenon and the properties of strains contained therein suggest that these phenanthrene degraders belong to the family *Enterobacteriaceae*, being most closely identified with the tribe *Klebsielleae* (5).

Phenon 7 contained two phenanthrene-degrading bacteria showing 75.5% similarity linking and were assigned to the genus *Vibrio*. Characteristically, strains of phenon 7 failed to utilize a large number of carbon sources, especially carbohydrates (Table 2).

Unclustered strains. Strain GID22 was assigned to the genus *Vibrio* owing to its sensitivity to 0/129 (10 μg), anaerogenic fermentation of glucose, motility, and oxidase reaction. Positive reactions were noted for arginine dihydrolase; lysine decarboxylase; *o*-nitrophenyl-β-D-galactopyranoside test; growth at 42°C; and acid from arbutin, amylase gelatinase, and DNase. Strain GID22 did not ferment sucrose, produce ornithine decarboxylase, or grow at 0 or 8% NaCl and was Voges-Proskauer negative. Strain GID30 was 0/129 resistant and anaerogenic; it fermented sucrose and produced arginine and ornithine decarboxylase, but failed to decarboxylate lysine. Characteristically, strains failed to utilize most amino acids as carbon sources but used the majority of carbohydrates. Strain GID34 most resembled an *Aeromonas* sp. in being 0/129 resistant, anaerogenic, Voges-Proskauer negative, and oxidase positive and decarboxylating only arginine. Strain GID34 grew in 0% NaCl but not at 42°C and did not produce elastase or lecithinase. Strain

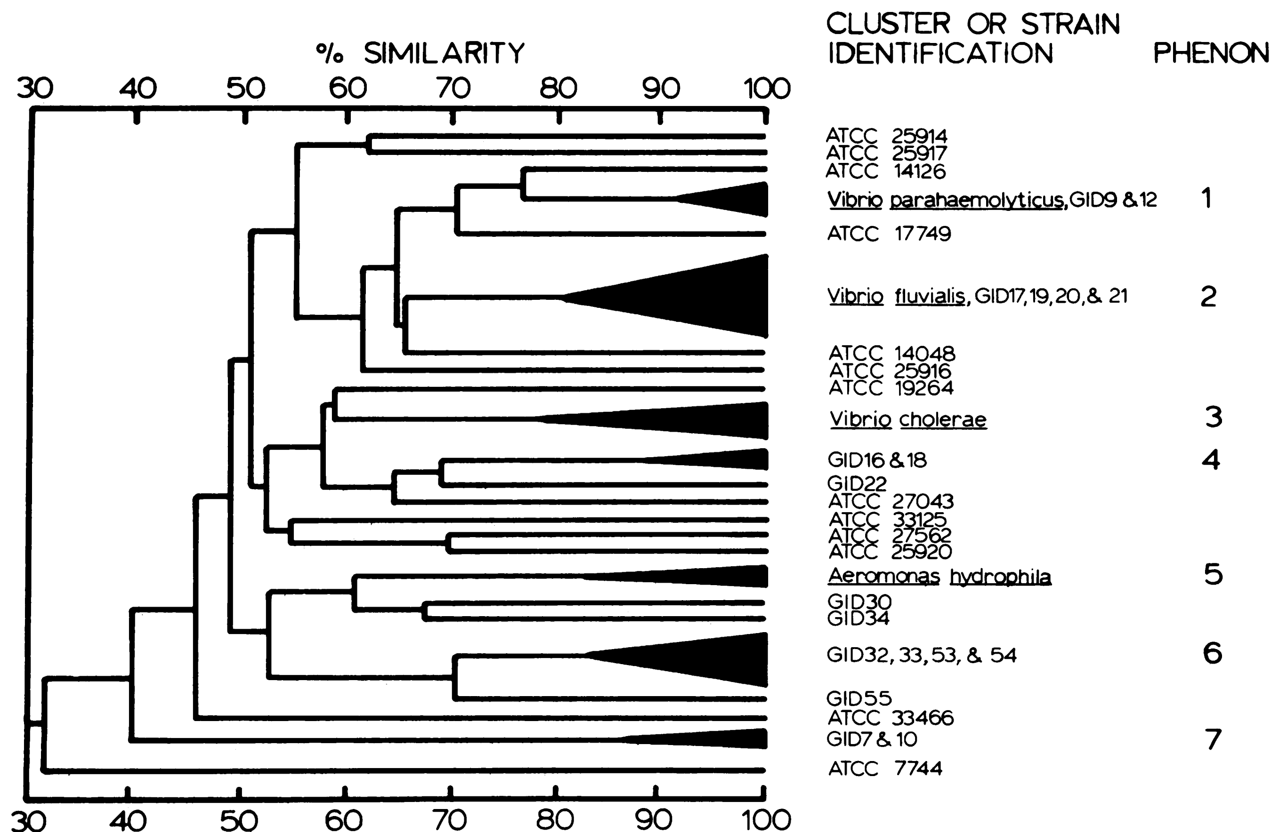

FIG. 1. Phenogram based on simple Jaccard (S_J) coefficient and unweighted-pair-group arithmetic average linkage.

TABLE 2. Properties of phena

Trait	% of following strains giving positive result (*n*):						
	Phenon 1 (3)	Phenon 2 (6)	Phenon 3 (3)	Phenon 4 (2)	Phenon 5 (2)	Phenon 6 (4)	Phenon 7 (2)
Growth in:							
0% NaCl	0	67	100	0	100	100	0
6% NaCl	100	100	0	100	0	100	100
8% NaCl	100	100	0	0	0	100	0
10% NaCl	0	0	0	0	0	0	0
Growth at:							
4°C	0	0	0	0	100	100	0
10°C	100	83	33	50	100	100	0
35°C	100	100	100	100	100	100	100
42°C	67	50	100	100	0	100	50
Oxidase	100	100	100	100	100	0	100
Nitrate reduction	100	100	100	100	100	100	100
Urease	0	0	0	0	0	100	50
ONPG[a]	67	100	100	100	100	100	0
Voges-Proskauer	0	0	100	0	50	75	0
Motility	100	100	100	100	100	75	100
Indole	100	100	100	100	100	75	100
Gluconate oxidation	0	0	0	0	0	0	0
Thornley arginine	0	83	0	0	100	100	50
Arginine decarboxylase	0	100	0	0	100	100	50
Lysine decarboxylase	100	17	100	100	50	0	0
Ornithine decarboxylase	100	0	100	100	0	100	0
Growth at pH 4.5	0	50	0	0	0	100	0
Chicken cell agglutination	0	0	66	100	100	0	0
Hemolysis	0	17	33	0	50	0	0
Luminescence	0	0	0	0	0	0	0
Catalase	100	100	100	100	100	100	100
Swarming	100	0	0	0	0	0	0
Gas from glucose	0	17	0	0	50	100	0
Acid from:							
Arbutin	0	33	0	100	50	75	100
Sucrose	0	100	100	50	100	100	100
Cellobiose	100	67	100	100	50	100	100
Mannose	100	83	100	100	100	100	100
Mannitol	100	83	100	0	100	100	100
Sorbitol	0	50	0	0	0	100	0
Salicin	0	50	0	100	50	100	100
Arabinose	100	100	0	0	100	50	0
Resistance to:							
0/129 (10 μg)	100	100	33	0	100	100	100
0/129 (50 μg)	100	100	0	0	100	75	0
0/129 (150 μg)	67	33	0	0	100	50	0
Novobiocin (5 μg)	100	100	0	0	100	100	100
Streptomycin (10 μg)	33	17	0	100	50	25	50
Ampicillin (10 μg)	100	100	0	0	100	0	100
Polymyxin B (50 IU)	100	0	33	100	0	0	100
Brilliant green (0.002%)	0	17	0	0	0	75	0
Crystal violet (0.002%)	0	0	0	0	50	100	0
Tellurite (0.0005%)	67	83	67	100	0	0	100
Methylene blue (0.01%)	67	100	0	0	100	100	0
Methyl violet (0.0002%)	100	100	33	100	100	100	50
Pyronin Y (0.002%)	0	17	0	0	100	100	0
Amylase	100	83	100	100	100	0	100
Gelatinase	100	100	100	100	100	0	100
Elastase	0	0	67	100	0	0	0
Lecithinase	100	100	100	100	0	0	100
Tween 80 hydrolysis	100	100	100	100	100	25	100
Tyrosine degradation	100	83	0	0	50	25	100
Alginase	0	0	0	0	0	0	0
DNase	100	100	100	100	100	25	100
Phosphatase	100	100	67	100	100	50	100
Sulfatase	0	0	0	0	0	0	0
Albumin hydrolysis	0	83	100	100	0	0	0
Chitinase	100	100	100	100	100	0	50
Chondroitinase	33	17	0	0	0	0	50
Casein hydrolysis	100	100	100	100	100	0	100

Continued on following page

TABLE 2—*Continued*

Trait	% of following strains giving positive result (*n*):						
	Phenon 1 (3)	Phenon 2 (6)	Phenon 3 (3)	Phenon 4 (2)	Phenon 5 (2)	Phenon 6 (4)	Phenon 7 (2)
Utilization as sole carbon source:							
Acetate	100	100	100	100	50	100	0
Citrate	100	100	100	100	50	100	50
Fumarate	100	100	100	100	50	100	50
Glutarate	0	50	0	0	0	0	0
DL-Glycerate	100	100	67	100	100	0	0
DL-3-Hydroxybutyrate	0	100	0	50	0	75	0
α-Ketoglutarate	100	100	100	50	100	0	0
DL-Lactate	100	100	0	0	0	100	0
Malate	0	100	0	0	100	100	0
Malonate	0	0	0	0	0	25	0
Propionate	33	100	0	0	0	0	0
Succinate	100	100	0	50	100	100	0
D-Alanine	100	100	0	100	0	100	100
L-Alanine	100	100	33	100	0	100	100
γ-Aminobutyrate	0	100	0	0	50	0	0
δ-Aminovalerate	0	17	0	0	0	0	0
L-Arginine	100	100	0	100	50	0	100
L-Asparagine	100	100	100	100	100	100	100
L-Aspartate	100	100	67	100	100	100	100
L-Citrulline	100	83	0	0	0	0	100
L-Glutamate	100	100	100	100	50	100	100
L-Glycine	100	33	0	0	0	0	100
L-Histidine	100	100	100	100	100	100	50
L-Hydroxyproline	67	17	0	50	0	0	0
L-Leucine	100	0	0	0	0	0	0
L-Ornithine	100	100	0	100	0	0	100
L-Proline	100	100	100	100	100	100	100
L-Serine	100	100	67	0	100	100	0
L-Threonine	100	100	33	100	50	0	100
L-Tyrosine	33	67	0	0	0	0	0
D-Amygdalin	0	33	0	0	0	0	0
L-Arabinose	100	100	0	0	100	100	0
Arbutin	0	33	0	0	0	75	0
Cellobiose	100	33	0	100	50	100	0
D-Fructose	100	100	100	100	100	100	0
D-Galactose	100	100	100	100	100	100	0
D-Gluconate	100	100	100	100	100	100	0
Glycogen	100	100	100	100	100	100	0
Lactose	0	0	0	0	0	100	0
Maltose	100	100	100	100	100	100	0
D-Mannose	100	83	67	100	100	100	0
Melibiose	0	17	0	50	0	25	0
Ribose	100	100	0	100	100	100	0
Salicin	0	83	0	100	0	100	0
Sucrose	0	100	100	50	100	100	0
Trehalose	100	100	100	100	100	100	50
Xylose	0	0	0	0	0	75	0
N-Acetyl-D-glucosamine	100	100	100	100	100	100	0
D-Glucosamine	100	100	100	100	0	100	0
Putrescine	100	67	0	0	0	25	0
Gluconate	100	67	0	100	0	100	0
Galacturonate	0	100	0	0	0	100	0
Sarcosine	0	0	0	0	0	0	0
Taurine	0	0	0	0	0	0	0
D-Arabitol	0	67	0	0	0	0	0
Ethanol	100	100	0	0	0	0	0
Glycerol	100	100	100	100	100	100	0
m-Inositol	0	0	0	0	0	100	0
Mannitol	100	100	100	50	100	100	0
l-Propanol	100	100	0	0	0	0	0
Sorbitol	0	50	0	0	0	100	0

GID55 was also placed in the genus *Aeromonas* because it was 0/129 resistant, aerogenic, and Voges-Proskauer positive but failed to grow at 42°C. Strain GID55 decarboxylated only arginine and lysine.

DISCUSSION

Phenanthrene-degrading strains belonging to the family *Vibrionaceae* were identified in this study by numerical

taxonomic techniques. Traditionally, bacterial phenanthrene degradation has been associated not with this family but with the family *Pseudomonadaceae* (9). From the primary isolation data, it appears that *Vibrionaceae* strains capable of degrading phenanthrene predominate over pseudomonads under conditions of chronic hydrocarbon pollution of marine and estuarine waters. Similar findings have been noted by Hada and Sizemore (7), who showed a high incidence of plasmid-carrying, presumptive *Vibrio* spp. in waters polluted from oil field platforms. Grimes et al. have also observed a predominance of *Vibrio* spp. in waters receiving anthropogenic contaminants (D. J. Grimes, F. L. Singleton, and R. R. Colwell, J. Appl. Bacteriol., in press). The possibility that phenanthrene degradation is plasmid mediated in the *Vibrio* strains examined in the present study is under investigation, and preliminary results of curing experiments suggest plasmid involvement (G. C. Okpokwasili and R. R. Colwell, manuscript in preparation).

Various microorganisms have been shown to mediate decomposition of phenanthrene in aquatic and soil environments. Specific taxa within the genus *Vibrio* have now been shown for the first time to be contributors to microbial degradation of phenanthrene in marine and estuarine environments. In addition, the *Vibrio* strains described here are unlike the luminescent *Vibrio* sp. described by Kiyohara and Nagao in their study of phenanthrene and naphthalene catabolism (8). The potential of *Enterobacteriaceae* family members to degrade phenanthrene is also described here and warrants further investigation.

Although many of the microbial and enzymatic pathways for phenanthrene degradation in the natural environment have been elucidated (8, 15), several studies are marred by inadequate characterization of bacteria involved in the processes. For example, identification of phenanthrene-degrading *Pseudomonas* sp. (21, 22) and *Vibrio* spp. (8) was accomplished by a few tests which, with more recent taxonomic developments, would not now be considered adequate for thorough and complete identification.

The use of numerical taxonomic principles and techniques for characterization of strains is relatively simple and inexpensive and should eliminate misidentification, as well as yield data for genetic manipulation studies, namely, marker characteristics for plasmid or chromosomal gene exchange. The role of plasmids in phenanthrene degradation is presently under study and will be separately communicated as part of a longer-term study of the potential of genetic engineering in biodegradative processes in marine and estuarine environments.

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LITERATURE CITED

1. **Barnsley, E. A.** 1983. Bacterial oxidation of naphthalene and phenanthrene. J. Bacteriol. **153**:1069–1071.
2. **Barry, M., and F. P. Yevich.** 1975. The ecological, chemical, and histopathological evaluation of an oil spill site. Mar. Pollut. Bull. **6**:171–173.
3. **Brown, R. S., R. E. Wolke, S. B. Sailar, and C. W. Brown.** 1977. Prevalence of neoplasia in 10 New England populations of the soft-shell clam (*Mya arenaria*). Ann. N.Y. Acad. Sci. **298**:522–534.
4. **Colwell, R. R., and J. D. Walker.** 1977. Ecological aspects of microbial degradation of petroleum in the marine environment. Crit. Rev. Microbiol. **5**:423–445.
5. **Cowan, S. T.** 1974. Family I. *Enterobacteriaceae*, p. 290–293. *In* R. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
6. **Evans, W. C., H. N. Fernley, and E. Griffiths.** 1965. Oxidative metabolism of phenanthrene and anthracene by soil pseudomonad. Biochem. J. **95**:819–831.
7. **Hada, H. S., and R. K. Sizemore.** 1981. Incidence of plasmids in marine *Vibrio* spp. isolated from an oil field in the Northwestern Gulf of Mexico. Appl. Environ. Microbiol. **41**:199–202.
8. **Kiyohara, H., and K. Nagao.** 1978. The catabolism of phenanthrene and naphthalene by bacteria. J. Gen. Microbiol. **105**:69–75.
9. **Kiyohara, H., K. Nagao, K. Kouno, and K. Yano.** 1982. Phenanthrene-degrading phenotype of *Alcaligenes faecalis* AFK2. Appl. Environ. Microbiol. **43**:458–461.
10. **Kiyohara, H., K. Nagao, and R. Nomi.** 1976. Degradation of phenanthrene through *o*-phthalate by an *Aeromonas* sp. Agric. Biol. Chem. **40**:1075–1082.
11. **Lee, R. F., R. Sauerheber, and A. A. Benson.** 1972. Petroleum hydrocarbons: uptake and discharge by the marine mussel *Mytilus edulis*. Science **177**:344–346.
12. **Lee, J. V., P. Shread, A. L. Furniss, and T. N. Bryant.** 1981. Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). J. Appl. Bacteriol. **50**:73–94.
13. **Roubal, W. T., T. K. Collier, and D. C. Malins.** 1977. Accumulation and metabolism of carbon-14 labeled benzene, naphthalene and anthracene by young coho salmon (*Oncorhychus kisutch*). Arch. Environ. Contam. Toxicol. **5**:513–529.
14. **Sherga, M., M. Meskill, and C. D. Litchfield.** 1979. Analysis of methods for the quantitative recovery of bacteria sorbed onto marine sediments, p. 21–29. *In* C. D. Litchfield and P. L. Seyfried (ed.), American Society for Testing and Materials special publication no. 673. American Society for Testing and Materials, Philadelphia.
15. **Sherrill, T. W., and G. S. Sayler.** 1980. Phenanthrene biodegradation in freshwater environments. Appl. Environ. Microbiol. **39**:172–178.
16. **Shiaris, M. P., and J. J. Cooney.** 1983. Replica plating method for estimating phenanthrene-utilizing and phenanthrene-cometabolizing microorganisms. Appl. Environ. Microbiol. **45**:706–710.
17. **Sneath, P. H. A., and R. Johnson.** 1972. The influence on numerical taxonomic similarities of errors in microbiological tests. J. Gen. Microbiol. **72**:377–392.
18. **West, P. A., and R. R. Colwell.** 1984. Identification and classification of the Vibrionaceae—an overview, p. 285–364. *In* R. R. Colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.
19. **West, P. A., J. V. Lee, and T. N. Bryant.** 1983. Numerical taxonomy of strains of *Vibrio* species isolated from water and birds in Kent, England. J. Appl. Bacteriol. **55**:263–282.
20. **Walker, J. D., R. R. Colwell, M. C. Hamming, and H. T. Ford.** 1975. Petroleum hydrocarbons in Baltimore Harbor of Chesapeake Bay: distribution in sediment cores. Environ. Pollut. **9**:231–238.
21. **Wodzinski, R. S., and J. E. Coyle.** 1974. Physical state of phenanthrene for utilization by bacteria. Appl. Microbiol. **27**:1081–1084.
22. **Wodzinski, R. S., and M. J. Johnson.** 1968. Yields of bacterial cells from hydrocarbons. Appl. Microbiol. **16**:1886–1891.