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Pseudomonas halodurans sp. nov., a halotolerant bacterium

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Abstract. Twenty-nine (29) isolates (strains) were obtained from the Great Bay Estuary, New Hampshire, using a synthetic seawater medium with NaCl concentrations between 0.30 and 2.65 M. All the strains were gram-negative; did not accumulate or hydrolyze poly-β-hydroxybutyrate; demonstrated *ortho* ring cleavage on aromatic compounds and showed the following homogeneous characteristics: utilization of substrates, antibiotic response, acid production from sugars, colony morphology, catalase and oxidase production, lack of pigmentation and flourescence; and had a mean guanine-plus-cytosine content of deoxyribonucleic acid composition of 63.2 ± 1.1 mol %. The strains demonstrated flagellated dimorphism; being motile by a singular polar flagellum at NaCl concentrations up to 0.8 M, and nonmotile and aflagellated at higher NaCl concentrations. A total of 150 biochemical, cultural, morphological, nutritional, and physiological traits were tested for the 29 strains and the type strain of six pseudomonad reference species. The 29 strains and reference strains were then compared with other reference and nonreference strains of the following genera: *Aeromonas*, *Alcaligenes*, *Alteromonas*, *Arthrobacter*, *Flavobacterium*, *Pseudomonas*, and *Vibrio* using 125 unit characters and employing numerical taxonomy by the Jaccard (S_J) coefficient and single linkage clustering method. The 29 strains were similar at the 95% level and clustered with several known *Pseudomonas* strains between the 70–75% similarity level (S) but clustered separately at S greater than 75% level. A type strain has been deposited with the American Type Culture Collection (ATCC 29686) and has been named *Pseudomonas halodurans* sp. nov.

Key words: *Pseudomonas halodurans* – Isolation – Species description – Halotolerant bacterium

Halotolerance i.e., the ability to tolerate NaCl concentrations many times greater than encountered by an organism in its natural environment, is more common among terrestial than marine bacteria (MacLeod 1965). Since terrestrial bacteria may have envolved from marine bacteria (McLeod 1965), halotolerance very likely is common in the marine environment. However, most of the detailed studies of marine bacterial deal with requirement for, rather than with tolerance of, NaCl (MacLeod 1965, 1968). The limited data available suggest that NaCl concentrations somewhat higher than that of seawater (0.47 M Na) inhibit or arrest growth of many marine bacteria (Brown and Turner 1963; Cobet et al. 1970; MacLeod and Onofrey 1957; Tyler et al. 1960; ZoBell 1956). In contrast, some marine bacteria have been reported to tolerate up to 4.5 M NaCl (Forsyth et al. 1971; Shah and deSa 1964). Although extremely halotolerant bacteria do exist in the marine environment, optimal growth usually is reported to be at NaCl concentrations similar to those of the natural environment, suggesting marine bacteria, in general, to be stenohaline (MacLeod 1965, 1968).

In the course of investigating tolerance to NaCl of an estuarine bacterial population, halotolerant non-motile bacteria were isolated from a NaCl-supplemented seawater medium (unpublished results). In addition, three strains of motile bacteria were isolated from the unsupplemented seawater medium. The present study employed numerical taxonomy of phenetic characteristics, determination of deoxyribonucleic acid (DNA) base composition, and DNA/DNA hybridization to determine relationships between the non-motile and motile marine bacterial isolates and to provide confirmatory evidence for the proposal of *Pseudomonas halodurans* as a new species.

Materials and methods

Bacterial strains and media. The 29 strains were isolated from the Great Bay Estuary, Durham, New Hampshire. Samples (0.1 ml) of estuarine water collected between October, 1973 and November, 1974 were spread plated on an artificial seawater medium (ASWM) containing 1.5% Bacto-Agar (Difco, Detroit, MI, USA) (unless otherwise stated, all chemicals added as mass/vol.) and supplemented with NaCl concentrations ranging from 0.0 to 3.05 M [added on a mass by volume (mass/vol.) basis but reported as mol/l (M) NaCl]. Unsupplemented ASWM contained 0.30 M NaCl. ASWM supported colonial growth well since it also contained 0.1% Bacto-Peptone (Difco) and 0.1% Bacto-Yeast Extract (Difco). The artificial seawater was seven seas marine synthetic seawater (Utility Chemical Co., Paterson, NJ, USA). To approximate the salinity of the estuary and enhance colony enumeration, the salinity of the synthetic seawater was adjusted to 26 ± 1 parts per thousand and determined with an A & O salinometer (Goldberg, Model 10423). The bacterial population of the samples was enumerated after 14 days incubation at 20° C.

Abbreviations. ASWM, artificial seawater medium; BM, basal medium; BMA, based medium-agar; TTC, triphenyltetrazolium chloride; PHB, poly-β-hydroxybutyrate

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Table 1. Bacterial strains used

Organism/strain no.[a]	Reference strains	Strain no.[b]
29[e] *Pseudomonas halodurans* sp. nov. EO 30/73, EO 265/73, EN 225/73, EN 265/73, ED 30/73, ED 185/73 EJ 225/74, EJ 265/74, EF 30/74, EF 225/74, EF 265/74, EM 30/74, EM 225/74, EA 185/74, EA 265/74, EMa 225/74, EM 265/74, EJ 185/74, EJa 265/74, EJa 225/74, EJb 265/74, EA 225/74, EA 265/74, ES 30/74, ES 200/74, EO 185/74, EO2 265/74, ES 30/74, EN 185/74	1[e] *Aeromonas hydrophila*	ATCC 9071
	2 *Aeromonas hydrophila*	1S
	3 *Aeronomas hydrophila*	1R
	4 *Aeromonas salmonicida*[c]	ATCC 14174
	5 *Aeromonas* spp.	R1–R8
	6 *Alcaligenes* spp.	732, 985–997
	7 *Alteromonas* spp.	75–80
	8 *Arthrobacter crystallopoietes*[c]	ATCC 15481
	9 *Flavobacterium* group IIb	NCTC 10795
	10 *Flavobacterium* group IIb	NCTC 10796
	11 *Flacobacterum* group IIf	NCTC 10798
	12 *Flacobacterium* group IIf	NCTC 10799
	13 *Pseudomonas aeruginosa*[c, d]	ATCC 10145
	14 *Pseudomonas aureofaciens*[c]	ATCC 13985
	15 *Pseudomonas bathycetes*[c]	ATCC 19372
	16 *Pseudomonas beijerinckii*[c]	ATCC 19372
	17 *Pseudomonas coenobios*[c, d]	ATCC 14402
	18 *Pseudomonas doudoroffii*[c]	ATCC 27123
	19 *Pseudomonas elongata*[c, d]	ATCC 10144
	20 *Pseudomonas marina*[c, d]	ATCC 27129
	21 *Pseudomonas mendocina*[c]	ATCC 25411
	22 *Pseudomonas multivorans*[c]	ATCC 17759
	23 *Pseudomonas nigrifaciens*[c, d]	ATCC 19375
	24 *Pseudomonas perfectomarinus*[c, d]	ATCC 14405
	25 *Pseudomonas putida*[c]	ATCC 12633
	26 *Pseudomonas* spp. A	120–132
	27 *Pseudomonas* spp. B	22–30
	28 *Pseudomonas* spp. C.	140–150
	30 *Vibrio anguillarum*	ATCC 19264
	31 *Vibrio fisheri*[c]	ATCC 25918
	32 *Vibrio marinus*[c]	PS207
	33 *Vibrio* sp.	165–170

[a] Isolated from the Great Bay Estuary between October, 1973 and November, 1974 on synthetic seawater medium supplemented with NaCl concentrations from 0.30 to 2.65 M

[b] University of Maryland, College Park, MD, USA, collection unless otherwise noted; ATCC, American Type Culture Collecting, Rockville, MD, USA; NCTC National Collection of Type Cultures, London, England

[c] Type or suggested type strain

[d] Organisms obtained from the ATCC and tested with the 29 strains of *P. halodurans*

[e] Computer number

Bacterial colonies were picked from agar plates containing 1.85, 2.25, and 2.65 M supplemental NaCl. To determine which colonies on ASWM gave rise to the halotolerant colonies, the replica plate technique (Lederberg and Lederberg 1952) was used. Colonies that corresponded to colonies at superimposable sites on the NaCl-supplemented ASWM were picked from the master plates. In this way, potentially halotolerant organisms were indirectly selected. Five strains were isolated indirectly and 24 strains came directly from the NaCl-supplemented ASWM. These five isolates are representative of the type strain of the species.

To assure a precise composition of cations and anions a second artificial seawater medium was prepared with Lyman and Fleming (L and F) synthetic seawater (Lyman and Fleming 1940) diluted to 26 ± 1 parts per thousand. When this synthetic seawater contained 0.1% Bacto-Peptone and 0.1% Bacto-Yeast Extract it was designated the basal medium (BM). This medium was also supplemented with NaCl to yield final concentrations of 0.50 to 3.05 M. Basal medium with no supplemental NaCl contained 0.30 M NaCl. BM with 15 g of Bacto-Agar per liter was designated basal medium-agar (BMA).

The 29 strains of *P. halodurans* were compared to 107 previously analyzed strains and reference strains from the American Type Culture Collection (ATCC) and, the University of Maryland Culture Collection and represented species of the genera *Aeromonas*, *Alcaligenes*, *Alteromonas*, *Arthrobacter*, *Flavobacterium*, *Pseudomonas*, and *Vibrio* (Table 1). Wherever possible the type or suggested type strain was used.

Bacteriological investigations. Cultural characteristics of 29 strains of *P. halodurans* (Table 2), and single type strains of *P. aeruginosa*, *P. marina*, *P. coenobios*, *P. elongata*, *P. nigrifaciens*, and *P. perfectomarinus* were observed (unless otherwise stated) at 20° C with either mid-log phase aerated organisms or plates incubated for 72 h and were determined using the criteria of Colwell and Wiebe (1970), Meynell and Meynell (1970), Skerman (1967), The Manual of Microbiological Methods (1957), Stanier et al. (1966), Shewan et al. (1954), King et al. (1954), Squros (1955), and Hugh and Leifson (1953). Media were prepared and cells washed with L and F synthetic seawater with supplemental NaCl concentrations corresponding to those from which the organisms

Table 2. Characteristics of *Pseudomonas halodurans*

All strains (29) positive

Straight rods	Utilization as sole	Oxidative in Hugh and Leifson
Ring cleavage[a]	Carbon and energy source:	O–F medium
Gram negative	(a partial list)	Acid from:
Aerobic growth	Glucose	(a partial list)
Catalase	Mannose	Glucose
Oxidase	Galactose	Lactose
NaCl tolerance (12%)	Fructose	Sucrose
KCN tolerance (0.0075%)	Lactose	Galactose
TTC[b] tolerance (0.1%)	Citrate	Fructose
NO_3 reduced	Acetate	Salincin
NH_3 utilized	Succinate	Ribose
NO_3 utilized	α-Ketoglutarate	Lysine decarboxylase
	Benzoate	Lipase (Tween 80)
	L-Asparagine	Arginine dihydrolase
	L-Lysine	Phosphatase
	Glycerol	Resistant to:
		Trisulfa 10 μg
		Penicillin 10 IU
		Chloramphenicol 30 μg
		Vibriostatic agent (0/129) 1 μg/ml
		Growth at 4°–35° C
		Growth at pH 5.5–8.5

All strains (29) negative

Acid-fast strain	Utilization as sole	Acid from:
Anaerobic growth	Carbon and energy source:	(a partial list)
Fluorescence	Xylose	Rhamnose
Capsule	Arabinose	Xylose
Spore	Rhamnose	Mannitol
PHB[c] accumulation	Inulin	Inositol
PHB hydrolysis	Gluconate	Sensitive to:
Indole	Caproate	Tetracycline 30 μg
H_2S	Malate	Streptomycin 10 μg
Urease	β-hydroxybutyrate	Chloromycin 30 μg
Litmus milk	Mannitol	Polymyxin B 30 μg
Acetylmethylcarbinol	L-Leucine	Erthromycin 30 μg
Agar hydrolysis	L-Histidine	Nalidixic acid 30 μg
Gelatin hydrolysis	L-Tyrosine	Growth 0° C
Starch hydrolysis	Spermine	Growth at 40° C
Denitrification		Growth at pH 5.0
Creatinine utilization		Growth at pH 9.0

[a] Ring cleavage was *ortho* on aromatic compounds
[b] TTC, triphenyltetrazolium chloride
[c] PHB, poly-β-hydroxybutyrate
[d] Character coded for analysis by computer

were isolated. Unless otherwise stated, the tests were run for a maximum of 48 h and aerated by shaking. All chemicals were reagent grade and all tests were run with controls to determine false positive results.

The mechanism of aromatic ring cleavage, the screening of organic compounds as sources of carbon and energy, [employing replica plating (Lederberg and Lederberg 1952)], and the accumulation of poly-β-hydroxybutyrate (PHB), intracellularly and hydrolysis of PHB extracellularly were determinded according to Stanier et al. (1966). The polymer used for PHB hydrolysis and accumulation was obtained as purified granules isolated from *Bacillus megaterium* cells as described by Delafield et al. (1965).

DNA isolation, purification, and base composition from the 29 halotolerant strains of P. halodurans. DNA from late-logarithmic-phase cells of *P. halodurans* was extracted and purified according to Marmur (1961) modified to include phenol and predigested Pronase (Calbiochem Co, La Jolla, CA, USA) for 1 h at 37° C at 50 μg/ml (Colwell and Wiebe 1970). The solution of DNA was dialyzed overnight at 6° C against 0.001 M Na-EDTA in 0.22 M Na-phosphate buffer (pH 6.8). The DNA base composition, expressed as moles percent guanine plus cytosine (mol % GC) to total bases, was estimated from thermal denaturation temperatures (T_m) determined by the method of Marmur and Doty (1962) using the equation: mol % GC = 2.44 (T_m − 49.2) and from buoyant density measurements in CsCl gradients (Schildkraut et al. 1962) using the equation: mol % GC = 10.2 (p − 1.6616). Reference DNA from bacteriophage 41C (*Bacillus subtilis* host) at a density of 1.742 g/cm^3 was included in each gradient.

DNA/DNA hybridizations. Phosphorus-32 ($H_3\ ^{32}PO_4$, New England Nuclear, Boston, MA, USA) labeled DNA was obtained from mid-logarithmic-phase cells of 5 strains of *P. halodurans* and strains of *P. aeruginosa*, *P. coenobios* and *P. elongata* grown in BM and from 24 strains of *P. halodurans* grown in BM supplemented with NaCl concentrations from 1.85 to 2.65 M. DNA was extracted as previously described. The DNA hybridization experiments were performed using the conditions and techniques recommended by Denhardt (1966). After the 12-h incubation both sides of the filters were washed twice with 40 ml each of 2X SSC, incubated at 72° C, dried, placed in scintillation vials containing 10 ml of Aquasol (New England Nuclear), and counted in a Packard Tri-Carb 3330 Liquid Scintillation Spectrometer. Less than 5% of the labeled DNA was bound to the filters in the absence of the unlabeled DNA. The results are expressed as percent hybridization as compared to the annealing of unlabeled DNA and the corresponding homologous ^{32}P-DNA from each species taken as 100%.

Electron microscopy. Transmission electron micrographs were obtained from strains grown in BM to the late logarithmic growth phase. A drop of cell suspension was placed on 300-mesh Formvar, carbon-coated grids (Ladd Co, Burlington, VT, USA). The cells were negatively stained with 0.5% Na-phosphotungstate and examined using a Philips EM-200 electron miscroscope.

Computation of data. A total of 125 out of 169 characteristics were used in the numerical analysis. The remaining 44 characteristics were not used because all strains tested were either positive or negative. Characteristics were coded as "1" for positive or present, "0" for negative or absent, and "9" for noncomparable or not applicable. The data were analyzed using the Jaccard coefficient, S_j, which excludes negative matches (Sneath 1957). Clustering was by single linkage (Sokal and Michener 1958) from which a dendrogram was constructed. The programs used included the UMDTAXON 6 and IGPS program packages available on the University of Maryland UNIVAC 1106 computer. The final $n \times t$ matrix contained 136 organisms and 125 characteristics.

Results

Characteristics of the 29 strains of Pseudomonas halodurans. The 29 strains of *P. halodurans* isolated from the Great Bay Estuary were homogeneous in: being aerobic, gram-negative, catalase and oxidase positive, non-flourescent, not requiring complex growth factors, mechanism of ring cleavage, growing between 5 and 35° C and pH 5.5 to 8.5 (optimal growth occurring between 20 and 25° C and pH 6.5 and 8.0), not accumulating PHB intracellularly or hydrolyzing PHB extracellularly, acid production from carbohydrates, nutritional versatility, and antibiotic resistance (Table 2). Mean and standard deviations of the GC content of the DNA of the misspelled 29 strains of *P. halodurans* were 63.2 ± 1.1 mol %. Reassociation values of DNA for the 29 strains of *P. halodurans* employing the strain isolated on BMA as the labeled reference strain were 97.5 ± 1.7%.

The 5 strains of *P. halodurans* isolated from colonies grown on 0.30 M NaCl-ASWM and cultured in 0.30 BM NaCl-BM (pH 6.9) at 20° C, to the mid-logarithmic phase, were 0.4 to 0.6 μm by 1.5 to 2.0 μm and motile by means of a polar monotrichous flagellum (Fig. 1). The colonies on

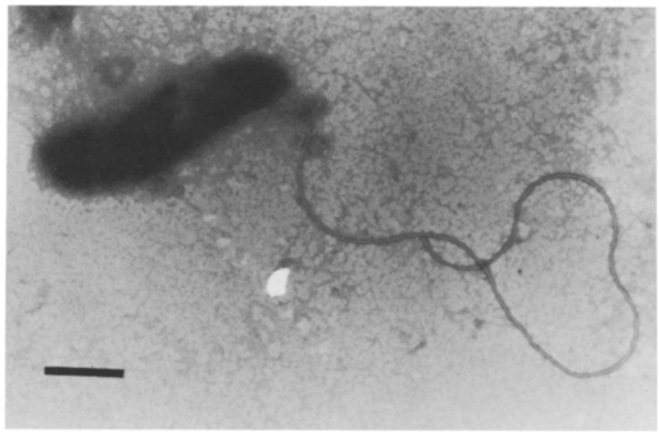

Fig. 1. Electron micrograph of the type stain of *P. halodurans* grown in BW with 0.30 M NaCl, exhibiting a single polar flagellum. Bar represents 0.5 μm

Extract Agar, Bacto-Marine Agar 2216, and BMA after 48 h at 20° C were nonpigmented, 2 to 3 mm in diameter, circular, smooth and entire and convex. The 24 strains of *P. halodurans* isolated from colonies grown on 1.00 to 2.65 M NaCl-ASWM and cultured in 1.00 to 2.65 M NaCl supplemented – BM were nonmotile, aflagellated, and measured 2.0 to 2.5 μm by 3.5 to 5.0 μm. These strains became motile and polarly flagellated when grown below 0.8 M NaCl (unpublished data).

The type strain of *P. halodurans* was similar to the type strain of the genus (*P. aeruginosa*) in: being flagellated, straight, motile, gram-negative rods, aerobic, catalase and oxidase positive, resistant to penicillin and vibriostatic agent 0/129 (2,4-diamino-6,7-diisopropyl pteridine) and having a mol GC content of DNA between 62 and 67% (Doudoroff and Palleroni 1974). The reassociation values of the DNA from *P. halodurans* and *P. aeruginosa* were 58.5 ± 2.0%. Further similarities with *P. aeruginosa* included mechanism of ring cleavage, lack of PHB accumulation and hydrolysis, nitrate reduction to nitrite, and production of lipase and arginine dihydrolase.

The type strain of *P. halodurans* differed from *P. aeruginosa* in: antibiotic sensitivity, organic compounds utilized as carbon sources, acid from sugars, fluorescent pigment, urease production, denitrification, gelatin hydrolysis, litmus milk coagulation, different growth response at 4° and 40° C, tolerance to 12% NaCl and 0.1% TTC, and lysine decarboxylase and phosphatase production (Doudoroff and Palleroni 1974). All 29 strains of *P. halodurans* demonstrated an obligate requirement for Na whereas *P. aeruginosa* did not.

The following traits differed between the 29 strains of *P. halodurans* and the type strains of known marine bacteria, *P. nigrifaciens*, *P. coenobios*, *P. elongata*, *P. perfectomarinus*, and *P. marina:* mechanism of ring cleavage, litmus milk reaction, and NaCl, KCN, and TTC tolerance (Baumann et al. 1972; Breed et al. 1957; ZoBell and Upham 1944). In addition, *P. nigrifaciens* was fluorescent, hydrolyed gelatin, but dit not; reduce nitrate to nitrite, utilize NO_3 as sole nitrogen source, produce lysine decarboxylase or arginine dihydrolase, and had complex mineral growth requirements and a mol % GC of 42.7. *P. coenobios* possessed two flagella, produced H_2S, hydrolyzed gelatin, did not reduce nitrate or use nitrate as sole nitrogen source, did not produce lipase, lysine decarboxylase, or arginine dihydrolase, and grew at

40° C. *P. elongata* possessed two flagella, produced a capsule and H_2S, hydrolyzed gelatin, agar, and starch, did not produce lipase, lysine decarboxylase, or phosphatase, and did not grow at 4° C. *P. perfectomarinus* was not motile, hydrolyzed starch, denitrified NO_3, did not; reduce nitrate, utilize creatine as sole carbon source, produce lipase, lysine decarboxylase, or phosphatase, or grow at 4° C, but grew at 40° C. *P. marina* possessed two flagella, was oxidase negative, accumulated and hydrolyzed extracellularly PHB, did not reduce nitrate or utilize NH_3 as sole nitrogen source, and did not produce lipase or lysine decarboxylase. Furthermore, the 29 strains of *P. halodurans* demonstrated unique patterns of acid production, utilization of compounds as sole carbon sources, and antibiotic sensitivities compared to the 5 other aerobic, marine pseudomonads. In addition, the reassociation values of the DNA from *P. halodurans* and *P. coenobios* and *P. elongata* were 62.8 ± 1.8 and 62.1 ± 1.2%, respectively.

The 5 type strains of *P. halodurans* were also compared with literature data for 34 strains of *P. nautica*, 23 strains of *Alcaligenes*, and 18 strains of *Alteromonas* (Baumann et al. 1972). Although *P. nautica* also showed an *ortho* mechanism of ring cleavage and did not accumulate/hydrolyze PHB, most or all strains denitrified KNO_3, utilized caproate, DL-malate, and DL-β-hydroxybutyrate as sole carbon sources, and grew at 40° C; most or all strains did not reduce nitrate, utilize glucose, mannose, fructose, sucrose, glycerol, benzoate, glycine, did not grow at 4° C and had a mol % GC content of DNA of 58% (well below that for *P. halodurans*). In fact, none of the strains of *P. nautica* were able to utilize any carbohydrates whereas *P. halodurans* demonstrated versatility in carbohydrate utilization. Although certain *Alcaligenes* spp. have mol % GC compatible to *P. halodurans*, they have peritrichous flagella, accumulate PHB, and demonstrate limited carbohydrate utilization (Baumann et al. 1972). Species of *Alteromonas* that have been identified, on the other hand, do not accumulate PHB and have a single polar flagellum, but have mol % GC ranging from 42 to 49 (significantly below that of *P. halodurans*), fix molecular nitrogen, lack a constitutive arginine dihydrolase, and show *meta* ring cleavage on aromatic compounds.

The 29 strains of *P. halodurans* were compared by computer with 107 previously analyzed and reference marine strains. The dendrogram indicates the clustering of 33 phena containing organisms isolated from soil, sediment, estuarine, and marine samples (Fig. 2). At similarity values (S) greater than 75%, the 29 strains of *P. halodurans* clustered separately from the other phena while at S values between 70 and 75%, the *P. halodurans* strains clustered with several known *Pseudomonas* strains, including named, culture collection strains.

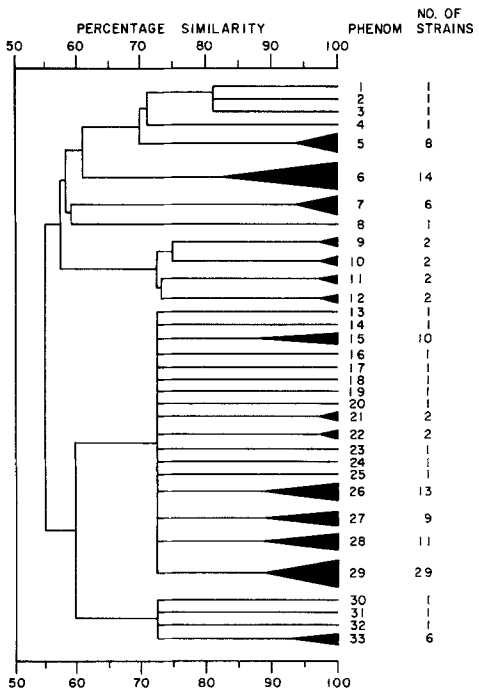

Fig. 2. Dendrogram indicating the arrangement of 136 strains after clustering by the single linkage and S_j coefficient method

Discussion

The results of the cultural tests indicated that the 29 strains of *P. halodurans* isolated from the Great Bay Estuary were physiologically similar and constituted a distinctive taxonomic group. These findings were corroborated by similarities among the strains in ring cleavage, mol % GC, PHB reaction, susceptibility to antimicrobial agents, utilization of organic compounds, acid production from sugars, DNA/DNA hybridization, and other taxonomic characteristics conducted at 26 parts per thousand and higher salinities. Furthermore, the computer analysis indicated that the 29 strains were similar at the 95% level. The 29 strains differed significantly from known reference strains in important taxonomic characteristics such as mechanism of ring cleavage, mol % GC of DNA, accumulation/hydrolysis of PHB and organic carbon utilization.

Many of the gram-negative, heterotrophic, motile marine bacteria that fall into groups established by numerical taxonomy have the general properties of the genera *Pseudomonas*, *Alcaligenes*, and *Altermonas* (Baumann et al. 1972). The initial subdivision between these genera can be based on the mechanism of ring cleavage, the mol % GC of DNA, and the accumulation and exogeneous hydrolysis of PHB (Stanier et al. 1966; Baumann et al. 1972). The mechanism of ring cleavage depends on distinctive metabolic pathways and enzymes for the degradation of a given aromatic compound and thus the mode of cleavage serves as an important taxonomic character (Stanier et al. 1966). Although the ranges of mol % GC for these genera are not firmly established, this genetic property of organisms is independent of culture conditions and is not known to be subject to either induced or spontaneous changes within the limits of analytical techniques (Mandel 1966) and thus also serves as an important taxonomic character. The accumulation of PHB is probably the best single character for the primary subdivision of the genus *Pseudomonas* (Stanier et al. 1966). Both the accumulation and exogenous metabolism of the PHB represents complex and distinctive biochemical and enzymatic processes that make these properties valuable taxonomic characters and thus many marine species isolated from salt environments can be quickly differentiated from *P. halodurans*.

The 29 strains of *P. halodurans* were similar to known and type strains of *Pseudomonas*. Thus the strains correspond to the description and definition of *Pseudomonas* given by Doudoroff and Palleroni (1974), Stanier et al. (1966), Skerman (1967), Colwell (1973), Colwell and Liston (1961a), and Shewan et al. (1960). They are strictly aerobic, gram-

negative rods, produce catalase, have a respiratory metabolism, are motile by means of a polar monotrichous flagellum (at NaCl concentrations below 0.80 M), utilize acetate and glucose, and have a GC content of their DNA's in the range of 58 to 70 mol %. These % values are in agreement with those for other pseudomonads (Mandel 1966). Further, DNA/DNA hybridization values between 50 and 70% are indicative of generic similarity (Colwell and Liston 1961a, b). Thus, the DNA/DNA reassociation values between the 29 halotolerant strains and other pseudomonads indicate generic relatedness but species diversity.

The numerical taxonomy data support further the identification of the 29 strains of *P. halodurans*. The *P. halodurans* strains clustered with several known culture collection and reference type strains at the 70 to 74% species level of similarity but clustered separately at a species level of similarity greater than 75% from the 107 other strains representing the 7 genera. A value of greater than 75% has been suggested as a possible species level of similarity (Colwell and Liston 1961a, b). Thus, the genetic data and numerical analyses provide strong evidence of membership within the genus *Pseudomonas*, but separate species status.

From the available descriptions of marine species of bacteria little information can be extracted for identification and classification purposes because many specific taxonomic tests were not done, the organisms are no longer in culture collections, descriptions are scanty, or media employed cannot by duplicated. Comparisons of data for the 29 strains of *P. halodurans* with the descriptions of other marine *Pseudomonas* spp. (especially *P. doudoroffii*, *P. elongata*, *P. nautica*, *P. nigrifaciens*, *P. marina*, and *P. perfectomarinus*) and other genera in the literature (Baumann et al. 1972; Breed et al. 1957; Colwell and Wiebe 1970; Doudoroff and Palleroni 1974; Dye and Lelliott 1974; Humm 1946; Shewan and Veron 1974; Shewan et al. 1960; Skerman 1967; Stanier et al. 1966; ZoBell and Upham 1944) do not reveal species level of similarity with the 29 strains of *P. halodurans*. Since the 29 strains of *P. halodurans* were compared to over 100 species and strains of marine and nonmarine organisms (Colwell and Gochnauer 1963; Colwell et al. 1967) and were found to be distinctly biochemically and physiologically different, it appears justifiable to propose *P. halodurans* as a new species and classified in the genus *Pseudomonas* for which the epithet *P. halodurans* (hal-o-dur-ans. Gr. n. *hals, halos* the sea, salt; L. v. *durare* to last, endure; M. L. masc. adj. *halodurans* intended to indicate NaCl endurance by the cells) is here proposed. The generic name complies with Rule 65 (no. 3) and recommendation 12 c of the International Code of Nomenclature of Bacteria (Lapage et al. 1975) and the specific epithet describes a property of the species – the ability to tolerate NaCl concentrations greater than 2.0 M. (Subsequent studies indicated that the halotolerant strains tolerated up to 3.75 M NaCl, unpublished data). Figure 1 depicts the type strain of *P. halodurans* (ATCC 29686).

P. halodurans demonstrated a remarkable ability to tolerate increased concentrations of NaCl, i.e., greater than 10-fold that of seawater. Because of its halotolerance, the organism may play a major role in nutrient recycling in environments, such as tidal pools, where a high NaCl concentration would be toxic to other bacterial species.

Description of the type strain of P. halodurans. A culture of strain *Pseudomonas halodurans* has been deposited with the American Type Culture Collection under the number ATCC 29686. The description of the type strain is the same as that of the species.

Taxonomic characteristics. The type strains of *P. halodurans* occur as single, straight rod cells measuring 0.4–0.6 µm in width by 1.5–2.0 µm in length and motile by means of a single polar flagellum (8–10 µm in length and 12–14 nm thick). Colonies are circular, convex, smooth and entire, nonpigmented, 2–3 mm in diameter on Extract and Bacto-Marine agar after 48 h at 20° C. The strains are heterotrophic with molecular oxygen as the universal electron acceptor. Optimal growth temperature is ca. 20° C with growth at 4° C. The GC content of the DNA is 63.2 ± 1.1 mol % (thermal denaturation and buoyant density). *P. halodurans* was isolated from estuarine water. Further taxonomic characteristics are listed in Table 2.

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