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Numerical Taxonomy of Moderately Halophilic Gram-negative Bacteria from Hypersaline Soils

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A total of 132 moderately halophilic bacteria were isolated from hypersaline soils with a Cl^- content between 2·36 and 12·72% (w/v) located near Alicante (S.E. Spain) and examined for 98 phenotypic characteristics including their response to cytological, physiological, biochemical and nutritional tests. They were submitted to a numerical analysis together with six reference strains using both simple matching (S_{SM}) and Jaccard (S_J) coefficients, and cluster analysis was carried out by the unweighted pair group method of association (UPGMA), single linkage and complete linkage. With the S_J coefficient and UPGMA clustering, eight phenons were obtained at the 65% similarity level. From each phenon representative strains were chosen for the determination of DNA base composition and for electron microscopy. Bacteria belonging to phenons D, E, and F were assigned to the genus *Alcaligenes*. Phenon G included 27 strains assigned to *Acinetobacter*, but the high G + C composition (58·9 mol %) of a representative strain of this phenon suggests that it may represent a new taxon. Phenons A, B, and C were designated *Flavobacterium* and phenon H was *Pseudomonas*. The bacteria found in these environments are not related to those from hypersaline waters or normal soils.

INTRODUCTION

Moderately halophilic bacteria have been defined as those showing optimal growth between 3 and 15% (w/v) NaCl (Kushner, 1978). Although very few groups of moderate halophiles have previously been described (Larsen, 1962; Kushner, 1978), we recently showed that a variety of taxonomic groups had moderately halophilic representatives in hypersaline waters (Ventosa *et al.*, 1982) and soils (Quesada *et al.*, 1982). The lack of systematic studies of isolates from natural sources has resulted in a scarcity of taxonomic information concerning moderate halophiles. Most of the descriptions refer to organisms isolated from salted food (Buchanan & Gibbons, 1974) or found as accidental laboratory contaminants (Novitsky & Kushner, 1976).

In hypersaline waters we found most of the genera that can be isolated from seawater: *Vibrio* was the most abundant, followed by *Flavobacterium, Alcaligenes, Alteromonas* and *Chromobacterium* (Ventosa *et al.*, 1982). All these bacteria were moderate halophiles having, in addition to a high (5–10%, w/v) salt requirement for optimal growth, a minimal requirement of 2–5% (w/v) NaCl in most cases. In hypersaline soils, however, although many halotolerant Gram-positive bacteria were found, most of the moderate halophiles were Gram-negative rods. These also required a high (about 5–10%, w/v) salt concentration for optimal growth, but a lower minimal salt concentration (0·5–2%, w/v) supported growth. These results probably reflect the ecological differences between water and soil environments, water being relatively homogeneous and constant whereas soil is heterogeneous and more affected by factors such as rainfall, which may eliminate over-specialized organisms (Quesada *et al.*, 1982).

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The purpose of this work was to compare moderate halophiles from soils with those isolated from waters, and also to provide a more complete taxonomy of this ecological group. Using numerical methods, we have studied 132 randomly-chosen new isolates of moderate halophiles, together with some reference strains from culture collections.

METHODS

Isolation and maintenance of strains. The micro-organisms were isolated from hypersaline soils near Alicante (S.E. Spain) which have been described previously in physical and chemical terms (Quesada *et al.*, 1982). Samples were taken as before at regular intervals from January 1980 to April 1981 (12 samplings from five different sites). The Cl^- content of the soils (determined using $AgNO_3$) was in the range 2·36 to 12·72%.

Isolation medium contained (%, w/v): yeast extract (Difco), 1; proteose-peptone no. 3 (Difco), 0·5; glucose, 0·1; bacto-agar (Difco), 2. This medium was supplemented with a balanced mixture of sea salts giving final concentrations of 0·5, 5, 10, 20, or 25% (w/v). The salts and proportions used were as in evaporated seawater according to Subov (Quesada *et al.*, 1982). One gram of each soil sample was suspended in 10 ml of a salt solution similar to those used for the culture media, tenfold dilutions were made (always keeping the same balanced salt concentrations) and 0·1 ml of each dilution was plated on the isolation medium of the same salt concentration. After incubation at 32 °C for 15 d in sealed plastic bags, three to five colonies per plate were randomly selected and subcultured on the same medium until pure. A total of 132 isolates of Gram-negative rods that were moderately halophilic, i.e. able to grow in media containing 2–20% (w/v) salts (the salt growth range determined as previously: Ventosa *et al.*, 1982), were selected for this study.

Reference strains. *'Chromobacterium marismortui'* ATCC 17056, *'Pseudomonas halosaccharolytica'* CCM 2851 and *Vibrio costicola* NCMB 701 were included. Quotation marks indicate species not in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980). Reference strains from a previous work on hypersaline waters (Ventosa *et al.*, 1982) were included: *Alcaligenes* sp. A-336, *Alteromonas* sp. A-387 and *Flavobacterium* sp. A-101.

Maintenance medium. The strains were maintained on slants of medium suitable for moderate halophiles (MH medium) containing 10% (w/v) marine salts. The final composition of this medium was (%, w/v): NaCl, 8·1; $MgCl_2$, 0·7; $MgSO_4$, 0·96; $CaCl_2$, 0·036; KCl, 0·2; $NaHCO_3$, 0·006; NaBr, 0·0026 (Rodriguez-Valera *et al.*, 1980) supplemented with 0·5% (w/v) proteose-peptone no. 3 (Difco), 1% (w/v) yeast extract (Difco) and 0·1% (w/v) glucose. This was solidified with bacto-agar (Difco) 2·0% (w/v) when necessary.

Characterization of isolates. The phenotypic characteristics (98) examined are given in Table 1. Most of the tests have been described elsewhere (Ventosa *et al.*, 1982); details of the remainder are given below. Incubation was at 32 °C for up to 7 d in sealed containers; all media contained 10% (w/v) marine salts and had their pH adjusted to 7·2 with 1 M-KOH (Rodriguez-Valera *et al.*, 1980). The ability to grow anaerobically was evaluated on plates of MH medium which were streaked and incubated in jars with the Gas Pak Anaerobic System (BBL). The appearance of growth was checked after 15 d incubation. The ability to grow in narrow Weinberg tubes of liquid MH medium containing glucose and phenol red (0·001%, w/v) was determined by rapid inoculation into the boiled and cooled medium. The tubes were sealed with sterile agar plugs and also covered with liquid paraffin. Phosphatase activity was tested by adding 1% (w/v) aqueous phenolphthalein diphosphate solution to MH medium.

Electron microscopy. Representative strains from each phenon were grown to mid-exponential phase on surface MH plate cultures covered with liquid medium. Samples of the liquid cultures were negatively stained with a 2% (w/v) solution of phosphotungstic acid (pH 7·0) and examined in a Carl Zeiss EM-9S-2 transmission electron microscope.

DNA composition. Exponential-phase cells of representative strains from each phenon were ruptured, and the DNA was purified using the method of Marmur (1961). The mol % G + C content was determined from the midpoint of the thermal denaturation profile (Marmur & Doty, 1962).

Numerical analysis. Taxonomic characters were coded in a binary form of the presence/absence type. Strain similarities were estimated with both simple matching (S_{SM}) (Sokal & Michener, 1958) and Jaccard (S_J) (Jaccard, 1908) coefficients, and cluster analysis was carried out using the unweighted pair group method of association (UPGMA), single linkage and complete linkage (Sneath & Sokal, 1973). Test error was estimated in eight repeated strains (Sneath & Johnson, 1972). Cophenetic correlation was also evaluated (Sneath & Sokal, 1973). These computations were performed using the MINT program of Dr F. J. Rolf of the Department of Ecology and Evolution, State University of New York at Stony Brook, N.Y., U.S.A., using a Univac 1108 computer in the Computer Centre of the University of Granada, Spain.

RESULTS

Morphological and halophilic characteristics

All the strains were Gram-negative rods and obligate aerobes. Conspicuous polyhydroxybutyrate inclusions were observed in the majority of strains.

All 132 strains grew optimally in 5–10% (w/v) salts media and are therefore moderate halophiles (Kushner, 1978). Most required at least 2% (w/v) salts for growth (Table 1). The detailed results of all the physiological, biochemical and nutritional tests are summarized in Table 1.

Numerical analysis

The results of the numerical study of the characteristics of the strains grouped by means of the S_J coefficient and UPGMA clustering yielded the dendrogram shown in Fig. 1. Simple matching coefficient (S_{SM}) and single linkage and complete linkage algorithms were also used, but cluster composition was not markedly affected by either the coefficient or the clustering method. Cophenetic values were 0·9726 and 0·8236 for the S_J and S_{SM} coefficients, respectively. The estimated test error was less than 3·0%, which would not significantly affect the cluster analysis.

The majority of the strains were grouped into eight phenons at a 65% similarity level. Eleven strains clustered separately, five of which were reference strains. Table 1 shows the features of the eight phenons, and Table 2 summarizes their differential characteristics.

Phenon A. This group included nine strains which were non-motile rods and oxidase positive. They reduced nitrates but not nitrites. Neither proteolytic, lipolytic nor amylolytic activity was shown. The G + C content of strain F8-11, chosen as representative of this group, was 69·4 mol %. These strains could be included in the genus *Flavobacterium* (Buchanan & Gibbons, 1974).

Phenon B. This phenon, containing only three strains, was very similar to phenon A (Table 1) but these strains reduced nitrites. Strain F8-6, taken as representative, had a G + C content of 61·8 mol %. This phenon could also be placed in the genus *Flavobacterium* (Buchanan & Gibbons, 1974).

Phenon C. This group included three strains which were non-motile short rods and oxidase positive. They reduced nitrate and nitrite and utilized a narrow range of compounds as sole source of carbon and energy. Strain G-4 was selected as representative of this group and had a G + C content of 65·9 mol %. These strains may be placed in the genus *Flavobacterium* (Buchanan & Gibbons, 1974).

Phenon D. This comprised 16 strains that clustered together because of their similar biochemical and nutritional characteristics; however, some were assigned to the genus *Flavobacterium* and others to *Alcaligenes*. Strains G-23 and F8-10, representatives of each group, respectively, had G + C contents of 65·4 and 69·2 mol %. Reference strain *Alcaligenes* sp. A-336 appeared in this phenon.

Phenon E. Twenty-four strains clustered in this group; all were straight, peritrichously flagellated rods. Most of the strains were oxidase and nitrate negative. None possessed proteolytic or amylolytic activities. Twelve of the strains were phosphatase positive, and this feature differentiated two subphenons related at different similarity levels. The two representative strains selected, G-30 (phosphatase positive) and F9-4 (phosphatase negative), had G + C contents of 66·7 and 67·4 mol %, respectively. Both subphenons can be assigned to *Alcaligenes* (Buchanan & Gibbons, 1974).

Phenon F. The 40 strains included here were long, motile rods with peritrichous flagellation (Fig. 1). They were oxidase positive but not proteolytic nor lipolytic. Some (30%) of the strains hydrolysed urea. Strain F5-7 had a G + C content of 66·7 mol %. All members of this phenon were assigned to the genus *Alcaligenes* (Buchanan & Gibbons, 1974).

Phenon G. This group comprised 27 strains that clustered at the 66% similarity level and constituted a very homogeneous group of predominantly short rods, occasionally with large curved cells and filaments (sometimes rather numerous). Most were non-motile, oxidase negative, H_2S producers and reduced nitrates but not nitrites. All were phosphatase positive.

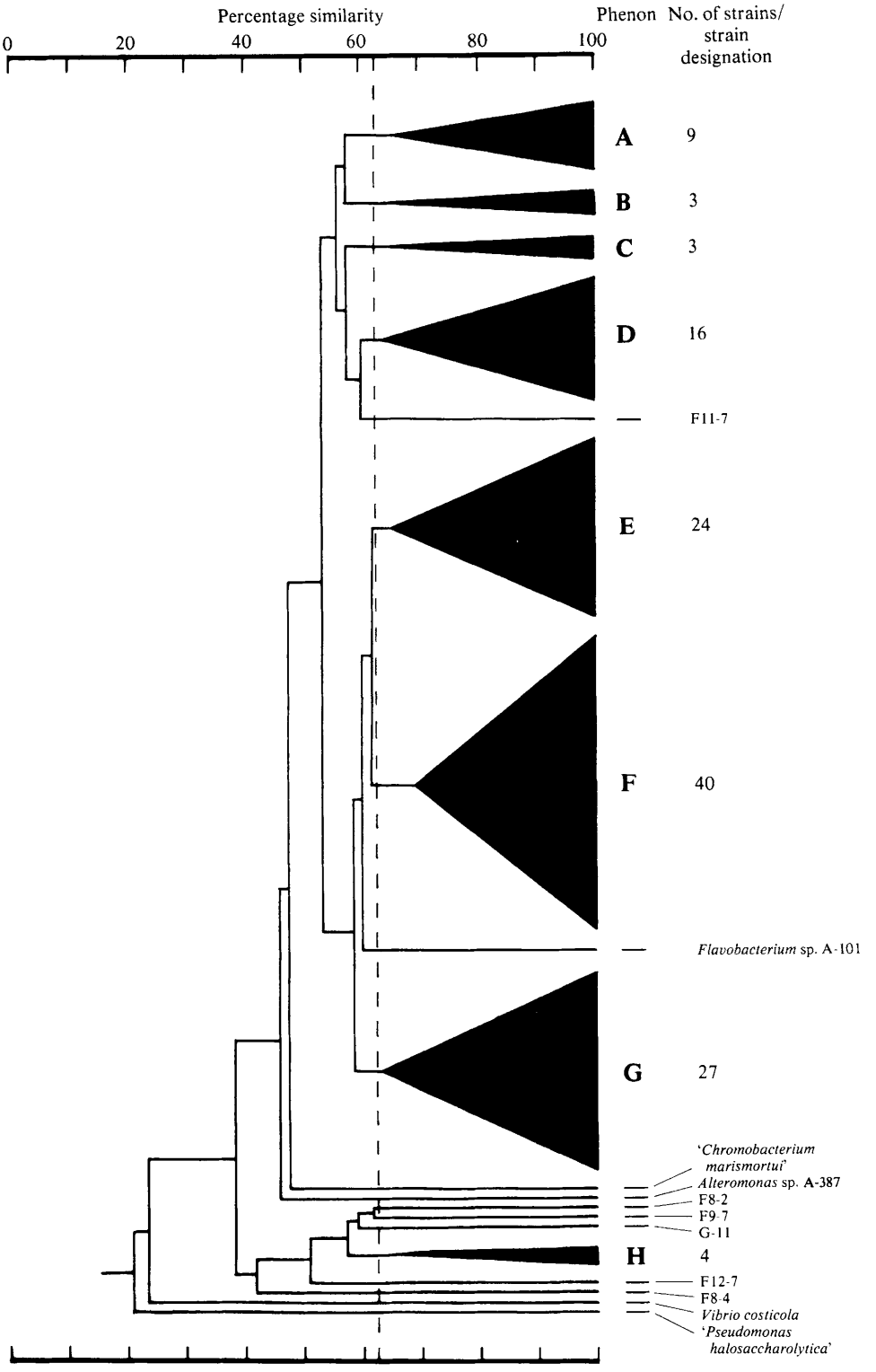

Fig. 1. Simplified dendrogram showing the clustering of strains into eight phenons based on the S_J coefficient and unweighted average linkage clustering (UPGMA), for 132 moderately halophilic Gram-negative bacteria isolated from hypersaline soils and six reference strains.

Table 1. *Frequencies of positive characters found in the eight phenons, expressed as a percentage of the total scored to each group for the given test*

Phenon ...	A	B	C	D	E	F	G	H
No. of strains ...	9	3	3	16	24	40	27	4
Morphology								
Long rods	44	0	0	63	96	100	4	100
Coccobacillary	56	100	100	37	4	0	96	0
Colony pigment								
Cream	66	33	0	44	88	78	67	0
Yellowish	34	66	100	56	12	22	7	100
Orange	0	0	0	0	0	0	26	0
Motility	0	0	0	56	96	95	22	100
Salts: growth at % (w/v)								
0·5	0	0	0	12	0	10	85	50
2	100	100	100	100	100	100	96	100
15	90	100	100	100	96	100	100	75
20	50	100	100	94	96	100	96	50
25	11	0	33	50	83	93	89	0
30	11	0	0	25	40	93	89	0
pH: growth at								
pH 5	0	0	33	0	0	47	41	0
pH 8	89	100	100	100	100	100	100	100
pH 9	54	100	100	100	96	93	100	100
pH 10	22	66	100	75	88	88	96	25
Temperature: growth at								
5 °C	33	0	100	50	50	10	70	0
15 °C	89	100	100	94	100	100	100	100
45 °C	11	0	0	56	50	25	48	0
Acid production from								
Galactose	11	33	0	12	75	93	4	75
Glucose	77	0	33	87	50	93	7	100
Lactose	0	0	0	0	0	2	0	0
Maltose	22	0	100	68	25	83	0	75
Mannitol	0	33	0	48	4	10	7	0
Sucrose	22	33	0	75	16	88	0	0
Xylose	0	0	33	0	12	22	4	75
Oxidase	89	100	100	100	20	80	4	100
H_2S production	33	33	0	0	76	80	89	50
Nitrate reduction to nitrite	100	100	100	69	20	65	93	100
Nitrite reduction to gaseous compounds	0	100	100	62	0	2	0	25
Casein hydrolysis	0	0	0	0	0	0	4	0
Gelatin hydrolysis	0	0	0	0	0	0	26	0
Starch hydrolysis	0	0	0	6	0	2	0	100
Tween 80 hydrolysis	0	0	0	6	0	0	0	100
Urea hydrolysis	0	0	0	0	0	30	0	0
Methyl red	0	0	0	0	4	0	0	0
Voges–Proskauer	0	0	0	0	4	0	0	0
DNAase	0	0	0	0	0	0	4	0
Phosphatase	0	0	0	0	54	0	100	0
Utilization of organic compounds as sole source of carbon and energy:								
Carbohydrates								
Aesculin	88	0	0	6	16	78	100	0
L-Arabinose	55	0	0	0	80	98	93	0
D-Cellobiose	11	0	0	0	20	90	100	0
D-Fructose	100	100	66	94	100	100	100	100
D-Galactose	66	33	0	31	100	98	100	0
Lactose	33	0	0	0	0	10	100	0
Maltose	100	66	100	100	92	90	100	100
D-Mannose	55	0	33	50	92	90	100	75
L-Raffinose	0	0	0	0	0	2	93	0
L-Rhamnose	0	0	0	12	54	15	89	0
Salicin	0	0	0	0	20	85	96	0
L-Sorbose	33	0	0	0	4	0	37	0

Table 1 (*continued*)

Phenon ...	A	B	C	D	E	F	G	H
No. of strains ...	9	3	3	16	24	40	27	4
Starch	55	0	0	0	32	0	4	100
Sucrose	77	100	66	100	92	98	100	25
D-Trehalose	77	66	100	100	100	95	100	25
D-Xylose	88	100	33	31	60	85	100	25
Alcohols								
Adonitol	88	0	0	0	0	2	44	0
Dulcitol	0	0	0	0	0	2	33	0
DL-Glycerol	88	66	100	87	96	90	100	75
meso-Inositol	88	66	0	31	64	0	96	25
D-Mannitol	44	33	0	44	50	35	74	0
D-Sorbitol	88	100	66	44	54	32	89	0
Carboxylic acids								
Acetate	88	100	66	100	96	95	96	75
Benzoate	0	0	0	6	28	95	0	0
Caprylate	0	0	0	0	0	2	18	0
Citrate	88	100	100	94	100	93	100	100
Formate	100	100	100	100	100	95	100	100
Fumarate	100	100	100	100	100	95	100	100
D-Gluconate	100	100	66	75	100	93	100	100
Hippurate	66	66	0	56	66	90	15	25
DL-Lactate	100	100	66	94	88	98	15	100
DL-Malate	100	100	66	100	100	98	15	100
Oxalate	0	66	0	100	8	10	11	0
Pyruvate	100	100	66	100	100	90	100	50
Propionate	88	100	66	94	100	98	100	100
Succinate	100	100	100	87	100	98	100	100
D-Tartrate	11	66	0	19	16	5	89	0
Utilization of amino acids as sole source of carbon, nitrogen and energy:								
L-Alanine	100	100	100	100	100	95	100	50
L-Arginine	88	66	66	94	100	95	100	25
L-Asparagine	100	100	100	100	100	98	67	100
L-Aspartic acid	100	100	100	94	100	93	100	50
L-Glutamic acid	100	100	100	100	100	95	100	100
L-Histidine	88	0	0	81	96	15	100	0
L-Isoleucine	33	66	0	56	96	20	44	0
L-Leucine	33	100	0	81	80	25	52	0
L-Lysine	11	0	0	56	80	75	67	25
L-Ornithine	11	0	66	81	96	57	33	25
L-Serine	100	100	100	100	100	98	100	25
L-Tryptophan	0	33	0	6	24	27	7	25
L-Valine	0	33	100	25	0	2	7	0

All strains were Gram-negative, formed catalase, were strict aerobes, grew at 5 and 10% (w/v) total salts, grew at pH 6 and 7, grew at 25 and 32 °C, and grew on D-glucose as sole source of carbon and energy. None formed indol or grew on inulin or L-cysteine.

They were very versatile nutritionally, and were able to utilize a very high number of compounds as sole carbon and energy sources. The strains in this phenon were able to grow at low salt concentrations (0·5%, w/v) although for optimal growth they needed 5–10% (w/v) total salts. Strain F2-12 had a G + C content of 58·9 mol %. With the exception of G + C value these strains could be assigned to the genus *Acinetobacter* (Buchanan & Gibbons, 1974).

Phenon H. This phenon was composed of four strains, which were straight rods motile by one or a few polar flagella. Thin filaments were common. They were oxidase positive, reduced nitrates and showed amylolytic and lipolytic activities. Few organic compounds were used as sole carbon and energy sources. The representative strain F12-1 had a G + C content of 58·7 mol %. These four strains can be included in the genus *Pseudomonas* (Buchanan & Gibbons, 1974).

Table 2. *Differential characteristics of the eight phenons of moderately halophilic Gram-negative bacteria from hypersaline soils*

Phenon ...	A	B	C	D	E	F	G	H
No. of strains ...	9	3	3	16	24	40	27	4
Morphology								
Long rods	4	−	−	10	23	+	1	+
Coccobacillary	5	+	+	6	1	−	26	−
Motility	−	−	−	9	23	38	6	+
Flagella	−	−	−	−/per.	per.	per.	−/per.	pol.
Growth at 0·5% (w/v) salt concentration	−	−	−	2	−	4	23	2
Growth at 5 °C	3	−	+	8	12	4	19	−
Oxidase	8	+	+	+	5	32	1	+
H_2S production	3	1	−	−	19	32	24	2
Nitrate reduction to nitrite	+	+	+	11	5	26	25	+
Nitrite reduction to gaseous compounds	−	+	+	10	−	1	−	1
Starch hydrolysis	−	−	−	1	−	1	−	+
Tween 80 hydrolysis	−	−	−	1	−	−	−	+
Phosphatase	−	−	−	−	13	−	+	−
Utilization of:								
D-Cellobiose	1	−	−	−	5	36	+	−
Oxalate	1	2	−	+	2	4	3	−
L-Histidine	8	−	−	13	23	6	+	−
L-Leucine	3	+	−	13	19	10	14	−

+, all strains positive; −, all strains negative. Numbers indicate number of positive strains; underlined numbers indicate that the number represents 80% or more of the strains. per., peritrichous flagella; pol., polar flagella.

DISCUSSION

There are few published studies of bacteria from saline soils (Cervantes & Olivares, 1976; Mahmoud *et al.*, 1978), and taxonomic descriptions of such organisms are scarce. Henis & Eren (1963), working on a saline soil near the Red Sea, concluded that more colonies developed on media without salts than on media with 10% (w/v) NaCl. These results differ from ours, probably because these authors used media with NaCl as sole salt, while other ions such as Mg^{2+} and K^+ are also required for good growth of moderately halophilic bacteria (Kushner, 1978). As in our previous study on bacteria from hypersaline soils (Quesada *et al.*, 1982), most of the population living in the soils studied were moderate halophiles as defined by Kushner (1978), since they grew optimally between 5 and 10% (w/v) salts. This was also the case for bacteria from hypersaline waters (Rodriguez-Valera *et al.*, 1981; Ventosa *et al.*, 1982). Nevertheless, many bacteria isolated from hypersaline soils were able to grow at low salt concentrations, (about 0·5%, w/v), while bacteria from hypersaline waters could not. This was a common character of the Gram-positive bacteria described by Quesada *et al.* (1982); however, 31 of the Gram-negative strains included in this study also showed tolerance for low salt concentrations.

Although differences existed among the strains included in phenons A, B and C, all can be considered as representatives of the genus *Flavobacterium*, and the separation among them probably arose from their different capability to use organic compounds as sole carbon and energy sources. Within the genus *Flavobacterium*, the only moderately halophilic species described to date is *F. halmephilum* (Elazari-Volcani, 1940). The strains included in these phenons show some resemblance the description of this species, although they differ by their ability to produce acids from sugars, their proteolytic activity and their nutrient requirements. Besides, *F. halmephilum* has a G + C content of 45·6 mol % and therefore belongs to Section I of this genus, while our strains, with a G + C content in the range 61·8–69·4 mol %, are in Section II. Among the marine bacteria belonging to the genus *Flavobacterium* and included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), *F. oceanosedimentum* is the only one that resembles the strains that appear in this study, and it differs from them only by the production of acids from some sugars, the ability to reduce nitrates, and salt response; the

G + C content of *F. oceanosedimentum* is also very similar (67·5 mol %) to that of the strains in phenons A, B and C. Holmes & Owen (1979, 1981) have proposed a redefinition of the genus *Flavobacterium*, restricting this genus to the species with low G + C content (31–40 mol %). Since it is clear that flavobacteria of high G + C content are easily isolated from a wide variety of environments (Hayes & Mapp, 1981), more studies are necessary to decide if they could be accommodated in the genus *Empedobacter* as proposed by McMeekin & Shewan (1978).

The strains included in phenons E and F were assigned to the genus *Alcaligenes*. Phenon E was very similar to the description of the marine bacterium *Alcaligenes cupidus* (Baumann *et al.*, 1972) except in the ability to reduce nitrate to nitrite and their different salt range for growth. However, phenon F was composed of a large group of bacteria with properties very different from those of previously described species of *Alcaligenes*. According to the G + C content, they were related to the marine bacterium *A. pacificus* (Baumann *et al.*, 1972) but there were many physiological, biochemical and nutritional differences from this species. In our opinion, they could constitute a new taxon not previously described. However, more genetic and molecular data are necessary to support this suggestion.

The 27 strains that clustered in phenon G had morphological, physiological, biochemical and nutritional characteristics of the genus *Acinetobacter* (Buchanan & Gibbons, 1974). However, the G + C content of the representative strain of this group was 58·9 mol %, whereas the range accepted for this genus is 42–47 mol % (Henriksen, 1973). Moderately halophilic bacteria have not been described and accepted as members of this genus. Onishi & Hidaka (1978) studied the properties of an amylase produced by a moderately halophilic *Acinetobacter* sp. but a description of this strain has not been published. It seems clear that the strains in phenon G represent a new group of micro-organisms that could be included in *Acinetobacter*, but the different G + C content could indicate that they belong to not only a different species but possibly a different genus. Studies are in progress to determine if this difference in the G + C content is also reflected by differences at the phenotypic level.

Phenon H was included in the genus *Pseudomonas*, but these strains were not related to the moderate halophile *P. halosaccharolytica* used a marker in this study (Fig. 1). There were differences in nutritional versatility as well as in amylolytic, lipolytic and proteolytic activities. With respect to marine species of the genus *Pseudomonas* included in the Approved Lists (Skerman *et al.*, 1980), they could be related to *P. doudoroffii* (Baumann *et al.*, 1972), the difference being their lipolytic and amylolytic activities, absent in this species.

Comparison with previous studies (Ventosa *et al.*, 1982) suggests that the bacterial populations in the saline terrestrial environments studied here show many differences from bacteria from hypersaline waters. In fact, the reference strains from hypersaline waters did not group with the strains from the terrestrial habitat. All the strains studied here had a strict aerobic metabolism, a predominant feature that we had already reported for bacteria from hypersaline soils: very few strains were facultatively anaerobic (Quesada *et al.*, 1982). This point has been confirmed by an independent study carried out by Dr M. E. Rhodes-Roberts (personal communication). However, in hypersaline waters facultative anaerobes represent a major part of the population (Ventosa *et al.*, 1982). In aqueous saline environments *Vibrio* spp. were the most abundant group of moderately halophilic bacteria, whereas this genus is very scarce in hypersaline soils, which are dominated by the genera *Alcaligenes* and *Flavobacterium*. *Pseudomonas* is a very common inhabitant of normal soils (Alexander, 1961), but scarce in the hypersaline soils we studied. Nevertheless, the microflora of these hypersaline soils is much more similar taxonomically to the microflora of normal soils (Alexander, 1961) than it is to the microflora of hypersaline waters. This suggests that the general features of the environment may be more important than individual factors such as high salinity in determining the microflora of a particular habitat.

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