Two New Species of *Alteromonas*: *Alteromonas espejiana* and *Alteromonas undina*

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A total of 32 amino-acid-requiring, gram-negative, polarly flagellated, marine, rod-shaped bacteria were submitted to an extensive phenotypic characterization. Numerical analysis of the data grouped the strains into three clusters, one of which was identified as *Alteromonas haloplanktis*. The strains in the remaining clusters had properties characteristic of the genus *Alteromonas* and were phenotypically distinct from each other as well as from the previously characterized species of this genus. One cluster was designated *Alteromonas espejiana* sp. nov. (type strain, 261 [= ATCC 29659]), and the other was designated *Alteromonas undina* sp. nov. (type strain, 272 [= ATCC 29660]).

The genus *Alteromonas* was initially proposed for species of gram-negative, polarly flagellated, rod-shaped, strict aerobes of marine origin which had guanine-plus-cytosine (G+C) contents in their deoxyribonucleic acids (DNA) of 40 to 50 mol% (4). Organisms having these properties are common in the marine environment but appear to be rare or absent in terrestrial habitats. Originally the genus included four species: *A. communis*, *A. vaga*, *A. macleodii*, and *A. haloplanktis* (4, 15). All of these organisms utilize D-glucose and D-fructose by means of the Entner-Doudoroff pathway (1, 18). *A. communis* and *A. vaga* share a distinctive pattern of regulation of aspartokinase activity which differentiates them from marine species of *Pseudomonas* and *Alcaligenes* (2). Recently, Gauthier characterized three additional species of *Alteromonas*—*A. luteoviolaceus* (9), *A. rubra* (10), and *A. citrea* (11)—which produce violacein, prodigiosin, and a noncarotenoid lemon-yellow pigment, respectively. *A. rubra* resembles the prodigiosin-producing, polarly flagellated, strictly aerobic marine isolates that have been studied by Lewis and Corpe (14). All three species are distinctive in that they produce a number of antibiotics (8, 11-13).

During a study of growth factor-requiring marine bacteria in our laboratory, it was found that a number of strains shared phenotypic properties that allowed their placement into three distinct groups in the genus *Alteromonas*. One of these groups was identical to the species *A. haloplanktis*, whereas the other two groups represented new species of *Alteromonas*, which are described in the present report.

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MATERIALS AND METHODS

Bacterial strains. All of the isolates used in this study were obtained from seawater off the coast of Northern California by a direct isolation method previously described (4). Strain BAL-31 was a gift from R. M. Franklin.

Methods. The methods used for the phenotypic characterization of strains were previously described (3, 4). The sole modification consisted in the use of a basal medium containing (per liter) 1 mg each of L-alanine, L-arginine, L-asparagine, L-aspartate, L-cysteine, L-glutamate, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, and L-valine. The amino acids were filter sterilized and were added to the autoclaved medium, which had been cooled to 50°C. Since the sodium ion requirement of each organism was tested in this medium, all of the amino acids used were potassium salts or hydrochlorides. The determination of the effect of NaCl on growth rate and yield was performed as previously described (17). The G+C contents of the DNAs of selected strains were determined by the thermal denaturation method (16).

Enzyme assays. Cells were grown on the basal medium of Baumann et al. (3) supplemented with 0.25% yeast extract (Difco), 0.25% tryptone (Difco), and 0.3% D-glucose, D-fructose, or L-alanine and harvested during the exponential phase of growth. The methods used for the preparation of cell extracts and for the assays of 6-phosphofructokinase (EC 2.7.1.11), 1-phosphofructokinase (EC 4.2.1.12), and 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EC 4.2.1.14) have been described by Sawyer et al. (18).

RESULTS

A total of 32 strains of gram-negative, rod-shaped, strictly aerobic, marine bacteria were isolated which could grow in a medium containing 0.2% D-glucose, a mixture of amino acids, and an artificial seawater base. No growth occurred in media from which the amino acids

were omitted or in which the Na^+ of the artificial seawater was replaced by equimolar amounts of K^+ . None of the strains denitrified, reduced NO_3^- to NO_2^- , or grew at 40°C . A numerical analysis of the data grouped the strains, on the basis of their phenotypic similarities, into three clusters (Fig. 1). One cluster of six strains was phenotypically identical to previously characterized strains of *A. haloplanktis* (4, 15), and two strains of this cluster had G+C contents of their DNAs of 43.1 and 43.9 mol%, values similar to those previously found for other strains of this species. The phenotypic properties of the remaining two groups (designated J and K) are presented in Table 1. The G+C contents of the DNAs of strains 257 and 261 (group J) and 270 and 272 (group K) were 43.3, 43.1, 43.7, and 43.1 mol%, respectively. Both groups J and K were composed of strains that were motile by means of a single polar flagellum (Fig. 2 and 3). The cells from group J were straight rods (Fig. 4), whereas those from group K were curved (Fig. 5). The effects of NaCl concentration on the growth rate and cell yield of strains representative of groups J and K, determined in the marine medium previously described (17) which contained 0.2% D-glucose and the amino acid mixture, are presented in Fig. 6. The optimal concentrations for strains 261 and 272 were about 125 and 150 mM, respectively. Some of the properties distinguishing groups J and K from the previously characterized species of *Alteromonas* are presented in Table 2. Over 30 phenotypic traits distinguished groups J and K from *A. communis* and *A. vaga*, and between 4 and 15

traits distinguished these two groups from the remaining species of *Alteromonas*.

Cell extracts of strain 261 grown on D-glucose, D-fructose, or L-alanine, as well as extracts of strain 272 grown on D-glucose or L-alanine, lacked both 6-phosphofructokinase and 1-phosphofructokinase activities (≤ 0.002 mmol of substrate utilized per min per mg of protein). However, they did contain KDPG aldolase activity which, in L-alanine-grown cells, was 0.061 and 0.027 for strains 261 and 272, respectively. In the case of the former strain, the activity increased about twofold in D-glucose (0.127)- or D-fructose (0.111)-grown cells; a similar increase occurred in D-glucose (0.62)-grown cells of strain 272. These results suggest that, in these species, D-glucose and D-fructose are utilized via the Entner-Doudoroff pathway and not via the Embden-Meyerhoff pathway.

After the completion of the phenotypic characterization of groups J and K, we became aware of the possible similarity of strain BAL-31 of Espejo and Canelo (5) to isolates of group J. This strain has been shown to have a G+C content of about 43 mol% (7), a value similar to that of group J. A phenotypic characterization of BAL-31, restricted to the major diagnostic traits in Table 2, confirmed and extended the results of a previous characterization (5). Strain BAL-31 is an oxidase-positive, straight rod that is motile by means of a single polar flagellum, able to grow at 35°C but not 40°C , and produces an extracellular amylase, gelatinase, and lipase, but not an alginase or a chitinase. It is able to utilize D-galactose, sucrose, cellobiose, melibiose,

FIG. 1. Numerical analysis of strains of *Alteromonas*.

TABLE 1. *Phenotypic properties of A. espejiana and A. undina*^a

Trait	<i>A. espejiana</i> (group J)			<i>A. undina</i> (group K)		
	Pheno- type of species (18) ^b	Pheno- type of strain 261	Positive or negative strains	Pheno- type of species (8) ^b	Pheno- type of strain 272	Positive or negative strains
Production of:						
Amylase	+	+	(+): All strains	5	+	(-): 273-275
Gelatinase	+	+	(+): All strains	+	+	(+): All strains
Lipase	+	+	(+): All strains	+	+	(+): All strains
Alginase	+	+	(+): All strains	-	-	(-): All strains
Chitinase	-	-	(-): All strains	+	+	(+): All strains
Utilization of:						
L-Arabinose	-	-	(-): All strains	3	+	(+): 270-272
D-Glucose	+	+	(+): All strains	+	+	(+): All strains
D-Mannose	12	-	(-): 258-261,265,268	-	-	(-): All strains
D-Galactose	+	+	(+): All strains	-	-	(-): All strains
D-Fructose	14	+	(-): 254,257,260,268	-	-	(-): All strains
Sucrose	+	+	(+): All strains	+	+	(+): All strains
Trehalose	+	+	(+): All strains	7	+	(-): 276
Maltose	+	+	(+): All strains	+	+	(+): All strains
Cellobiose	9	+	(+): 253,255-261,265	-	-	(-): All strains
Melibiose	+	+	(+): All strains	-	-	(-): All strains
Lactose	+	+	(+): All strains	-	-	(-): All strains
N-Acetylglucosamine	-	-	(-): All strains	+	+	(+): All strains
Acetate	+	+	(+): All strains	+	+	(+): All strains
Propionate	+	+	(+): All strains	+	+	(+): All strains
Butyrate	16	+	(-): 265,268	6	-	(-): 272,273
Isobutyrate	17	+	(-): 268	+	+	(+): All strains
Valerate	13	+	(-): 251,260,265,266,268	+	+	(+): All strains
Isovalerate	16	+	(-): 265,268	-	-	(-): All strains
Pelargonate	+	+	(+): All strains	7	+	(-): 276
Caprate	+	+	(+): All strains	+	+	(+): All strains
Succinate	-	-	(-): All strains	+	+	(+): All strains
Fumarate	-	-	(-): All strains	+	+	(+): All strains
DL- β -Hydroxybutyrate	3	-	(+): 262-264	7	+	(-): 273
Citrate	17	+	(-): 268	-	-	(-): All strains
Pyruvate	+	+	(+): All strains	+	+	(+): All strains
Aconitate	17	+	(-): 268	-	-	(-): All strains
Mannitol	+	+	(+): All strains	-	-	(-): All strains
Ethanol	-	-	(-): All strains	+	+	(+): All strains
n-Propanol	-	-	(-): All strains	+	+	(+): All strains
n-Butanol	9	+	(+): 251-254,258-261,266	+	+	(+): All strains
Glycine	17	+	(-): 268	7	+	(-): 269
L- α -Alanine	+	+	(+): All strains	+	+	(+): All strains
L-Serine	+	+	(+): All strains	+	+	(+): All strains
L-Threonine	17	+	(-): 268	4	+	(+): 271,272,274,275
L-Leucine	15	+	(-): 262,264,268	+	+	(+): All strains
L-Isoleucine	9	+	(-): 255,257,260,262,264-268	3	-	(+): 271,274,275
L-Aspartate	17	+	(-): 268	-	-	(-): All strains
L-Arginine	15	+	(-): 262,266,268	2	-	(-): 271,273
L-Citrulline	16	+	(-): 258,259	2	-	(+): 273,276
L-Proline	17	+	(-): 267	6	+	(+): 269,270,272-275
L-Tyrosine	+	+	(+): All strains	+	+	(+): All strains
L-Phenylalanine	14	+	(-): 253,257,260,268	3	-	(+): 273-275
Growth at 4°C	-	-	(-): All strains	4	-	(+): 269-271,274
Growth at 35°C	8	-	(+): 252-254,256,262-264,267	-	-	(-): All strains

^a Symbols: +, all strains positive; -, all strains negative; numbers indicate number of positive strains; numbers in italics indicate that the number represents 80% or more of the strains.

^b Number of strains studied.

lactose, mannitol, and tyrosine but not D-mannose, salicin, D-gluconate, N-acetylglucosamine, succinate, fumarate, DL-glycerate, citrate, aconitate, erythritol, glycerol, or α -ketoglutarate. With the exception of alginase, citrate, and aconitate, all the other traits of BAL-31 match those of group J. Therefore, we conclude that BAL-31 is a member of this group.

DISCUSSION

The strains of groups J and K are strictly aerobic, polarly flagellated, gram-negative rods that have G+C contents of their DNAs of 43 to 44 mol%. All are of marine origin and require Na⁺ for growth. These properties indicate their placement in the genus *Alteromonas* (4). Since

FIG. 2 and 3. Leifson flagella stains of *A. espejiana* 261 and *A. undina* 272, respectively. Magnification, $\times 2,500$. Marker represents 5 μm .

FIG. 4 and 5. Phase-contrast micrographs of *A. espejiana* 261 and *A. undina* 272, respectively. Magnification, $\times 2,000$. Marker represents 5 μm .

groups J and K are phenotypically distinct from each other as well as from the previously characterized species of this genus (Table 2), we propose that these two groups be given new species designations. Group J is named *Alteromonas espejiana* sp. nov. (es.pe.ji.a'na. M. L. adj. *espejianus* of Espejo; named for R. T. Espejo, a Chilean microbiologist), and group K is named *Alteromonas undina* sp. nov. (un'di.na. L. fem.n. *undina* undine, water nymph).

A. espejiana contains straight rods (0.2 to 1.0 μm in width and 2.0 to 3.5 μm in length), each of which is motile by means of a single flagellum (Fig. 2 and 4). Its distinctive properties are the production of extracellular alginase, the ability to utilize D-galactose, sucrose, melibiose, lactose, citrate, aconitate, and mannitol, and its inability to utilize succinate, fumarate, or glycerol (Table 2). Strain 261 (= ATCC 29659) is the type strain of this species; its phenotypic properties, along with those of the species, are presented in Table 1. Strain BAL-31, which was characterized after the completion of this study, is a member of this species. This isolate is the host of a lipid-containing marine bacteriophage that has been the

object of extensive investigation (6).

A. undina consists of curved rods (0.7 to 0.9 μm in width and 1.8 to 3.0 μm in length), each of which is motile by means of a single flagellum (Fig. 3 and 5). Its distinctive properties are the production of an extracellular chitinase, the ability to utilize sucrose, *N*-acetylglucosamine, succinate, and fumarate, and its inability to utilize D-galactose, melibiose, lactose, citrate, aconitate, mannitol, or glycerol (Table 2). Strain 272 (= ATCC 29660) is the type strain of this species; its phenotypic properties and those of the species are presented in Table 1.

The recent studies of Gauthier (9–11), as well as the present phenotypic characterization of two new species of *Alteromonas*, suggest that this genus be subdivided into two major groups, one consisting of the phenotypically similar species *A. communis* and *A. vaga* and the second consisting of *A. macleodii*, *A. haloplanktis*, *A. luteoviolaceus*, *A. rubra*, *A. citrea*, *A. espejiana*, and *A. undina*. The species in the latter group share a number of properties of which the most striking is the ability to make a number of extracellular hydrolases, an attribute that is generally absent in species of marine strains of *Pseudomonas* and of *Alcaligenes* (4).

FIG. 6. Effect of NaCl on cell yield and growth rate of *A. espejiana* 261 and *A. undina* 272 tested in a marine medium (17). Symbols: ●, cell yield; ○, growth rate. All values have been normalized by making the maximal growth rate (doublings per hour during exponential growth rate) and yield equal to 100%.

TABLE 2. *Some distinguishing properties of species of Alteromonas^a*

Trait	<i>A. com- munis</i> ^b (33) ^c	<i>A. vaga</i> ^b (17)	<i>A. ma- cleodii</i> ^b (21)	<i>A. halo- plank- tis</i> ^d (25)	<i>A. espe- jana</i> (Group J) (18)	<i>A. un- dina</i> (Group K) (8)	<i>A. rubra</i> ^e (3)	<i>A. luteo- violaceus</i> ^f (16)	<i>A. citrea</i> ^g (3)
Mol% G+C of DNA	47	48	46	43	43	43	47	42	42
Straight rods ^h	—	+	+	+	+	—	+	+	+
Ring cleavage ⁱ	<i>m</i>	<i>m</i>	—	—	—	—	—	ND ^j	—
Oxidase	+	—	+	+	+	+	+	+	+
Growth at 35°C	+	+	+	21	8	—	+	+	1
Growth at 40°C	+	—	15	—	—	—	—	—	1
Amino acid(s) required:	—	—	—	6	+	+	+	ND	+
Production of:									
Amylase	—	—	19	4	+	5	+	15	+
Gelatinase	—	—	20	+	+	+	+	+	+
Lipase	—	—	+	+	+	+	+	+	+
Alginase	—	—	3	—	+	—	ND	ND	ND
Chitinase	—	—	—	16	—	+	—	—	1
Utilization of:									
D-Mannose	29	+	—	21	12	—	+	—	+
D-Galactose	11	15	+	5	+	—	—	ND	—
Sucrose	—	1	+	23	+	+	—	—	—
Cellobiose	—	14	+	—	9	—	—	—	—
Melibiose	—	—	20	—	+	—	ND	ND	ND
Lactose	—	—	+	1	+	—	—	—	—
Salicin	—	—	+	—	—	—	—	ND	—
D-Gluconate	+	+	18	1	—	—	—	ND	—
N-Acetylglucosamine	—	15	7	23	—	+	ND	ND	ND
Succinate	+	+	—	+	—	+	—	ND	—
Fumarate	+	+	—	+	—	+	—	ND	—
DL-Glycerate	4	—	+	—	—	—	ND	ND	ND
Citrate	+	+	—	21	17	—	—	ND	—
Aconitate	+	16	—	21	17	—	ND	ND	ND
Erythritol	—	+	—	—	—	—	—	ND	—
Mannitol	+	+	9	11	+	—	—	—	—
Glycerol	+	+	+	—	—	—	—	—	—
Ethanol	+	—	17	20	—	+	ND	ND	ND
γ-Aminobutyrate	+	16	—	—	—	—	ND	ND	ND
L-Tyrosine	—	—	18	23	+	+	ND	ND	ND
Sorbitol, DL-malate, α-ke- toglutamate, m-hydroxy- benzoate	+	+	—	—	—	—	—	ND	—
Other traits ^k	+	+	—	—	—	—	ND	ND	ND
Pigmentation	—	—	—	—	—	—	+ ^l	14 ^m	+ ⁿ

^a Symbols: +, all strains positive; —, all strains negative; numbers indicate number of positive strains; numbers in italics indicate that the number represents 80% or more of the strains.

^b Data from Baumann et al. (3).

^c Number in parentheses indicates number of strains studied.

^d Data from Baumann et al. (4), Reichelt and Baumann (15), and the present study.

^e Data from Gauthier (10).

^f Data from Gauthier (9, 10).

^g Data from Gauthier (11).

^h Symbols: +, straight rods; —, curved rods.

ⁱ Mechanism of aromatic ring cleavage by species capable of growth on aromatic compounds.

^j ND, Not determined.

^k Utilization of saccharate, meso-inositol, D-alanine, p-hydroxybenzoate, quinate, and sarcosine.

^l Prodigiosin.

^m Violacein.

ⁿ Lemon-yellow noncarotenoid pigment.

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REPRINT REQUESTS

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