Taxonomy of *Alteromonas*: *A. nigrifaciens* sp. nov., nom. rev.; *A. macleodii*; and *A. haloplanktis*

PAUL BAUMANN,^{1*} LINDA BAUMANN,¹ RON D. BOWDITCH,¹ AND BLAINE BEAMAN²

Department of Bacteriology, University of California,¹ and Department of Medical Microbiology, University of California Medical School,² Davis, California 95616

On the basis of phenotypic properties and the guanine-plus-cytosine content of the deoxyribonucleic acid, as well as the immunological relationship of the glutamine synthetase and the iron-containing superoxide dismutase, strain 217^T (= ATCC 19375) (T = type strain), which was previously designated "*Pseudomonas nigrifaciens*," was shown to be a member of a distinct species in the genus *Alteromonas*. Since this species designation was not included on the Approved Lists of Bacterial Names, it has been revived as *Alteromonas nigrifaciens* sp. nov., nom. rev., with type strain 217 (= ATCC 19375). The phenotypic properties of strains of *Alteromonas macleodii* and *Alteromonas haloplanktis* are also described.

In 1940 White described a bacterium which caused discoloration of mildly salted butter (7). This organism was straight, polarly flagellated, and rod shaped, grew at 4°C, required NaCl, and produced a brown or black pigment. White suggested that it be named "*Pseudomonas nigrifaciens*." Subsequently, Norton and Jones (4) isolated a pigmented marine strain which they equated with this species. In our studies of nonfermentative marine bacteria, we obtained a number of isolates having similar pigmentation (1). Characterization of these strains, as well as "*P. nigrifaciens*" strain 217^T (= ATCC 19375^T) (T = type strain), showed that they belong to different genera (1). In addition, the many differences between the phenotype of the "*P. nigrifaciens*" strain and the published phenotype of the isolate of Norton and Jones (4) indicated that these two strains could not be assigned to the same species. On the basis of the guanine-plus-cytosine (G+C) content of its deoxyribonucleic acid (DNA) (42.9 mol%) and its general phenotypic properties, "*P. nigrifaciens*" was shown to be a member of the newly proposed genus *Alteromonas* in 1972 (1). This species is not on the Approved Lists of Bacterial Names (6) and is listed as species incertae sedis in the chapter on *Alteromonas* in *Bergey's Manual of Systematic Bacteriology*, 9th ed. (P. Baumann, M. J. Gauthier, and L. Baumann, in press). A recent study of the relationships of the glutamine synthetases and iron-containing superoxide dismutases from species of *Alteromonas* indicated that "*P. nigrifaciens*" is closely related to *Alteromonas haloplanktis*, *Alteromonas espejiana*, and *Alteromonas undina* (E. F. DeLong, L. Baumann, R. D. Bowditch, and P. Baumann, Arch. Microbiol., in press). In this paper we revive this species under the designation *Alteromonas nigrifaciens* sp. nov., nom. rev. and provide a phenotypic description of the sole available strain of this species, as well as descriptions of *Alteromonas macleodii* and *A. haloplanktis*.

MATERIALS AND METHODS

"*P. nigrifaciens*" strain 217^T (= ATCC 19375^T), the original isolate of White (7), was obtained from the American Type Culture Collection. The sources of the remaining strains used in this study, as well as the methods used for isolation, have been described previously (1, 3). Detailed protocols of the methods used for phenotypic characterization of the strains have also been published previously (2).

The nutritional spectrum of "*P. nigrifaciens*" was determined in media containing 0.01% (wt/vol) yeast extract (2).

RESULTS

All of the strains of "*P. nigrifaciens*," *A. macleodii*, and *A. haloplanktis* were straight rod-shaped organisms which were motile by means of polar flagella. Figure 1 is an electron micrograph showing the general cell morphology and flagellation of "*P. nigrifaciens*." Figure 2 shows the colonial morphology and pigmentation of strain 217^T grown at different temperatures. Electron and light micrographs of *A. macleodii* and *A. haloplanktis* have been published elsewhere (5; Baumann, Gauthier, and Baumann, in press). All strains of these three species utilized D-glucose, maltose, acetate, propionate, and pyruvate as sole or principal sources of carbon and energy. All grew at 30°C, produced an extracellular lipase, were oxidase positive, and required Na⁺ or seawater for growth.

None of the strains was able to denitrify or to utilize H₂ as an energy source and CO₂ as a carbon source. None grew at 45°C, accumulated poly-β-hydroxybutyrate as an intracellular reserve product, or had a constitutive arginine dihydrolase system. The following compounds were not utilized by any of the strains: D-ribose, D-arabinose, D-fucose, L-rhamnose, inulin, saccharate, mucate, glucuronate, formate, oxalate, malonate, maleate, glutarate, adipate, pimelate, suberate, azelate, sebacate, DL-malate, D-(−)-tartrate, L-(+)-tartrate, meso-tartrate, α-ketoglutarate, laevullinate, citraconate, itaconate, mesaconate, erythritol, sorbitol, meso-inositol, adonitol, ethylene glycol, 2,3-butyleneglycol, methanol, isopropanol, isobutanol, D-mandelate, L-mandelate, benzoyl formate, benzoate, o-hydroxybenzoate, m-hydroxybenzoate, p-hydroxybenzoate, phenylacetate, quinate, D-α-alanine, β-alanine, L-norleucine, γ-aminobutyrate, δ-aminovalerate, L-tryptophane, D-tryptophane, DL-kynurenine, kynurenate, anthranilate, m-aminobenzoate, p-aminobenzoate, methylamine, ethanalamine, benzylamine, putrescine, spermine, histamine, tryptamine, butylamine, 2-amylamine, phenylamine, betaine, sarcosine, creatine, pantothenate, acetamide, nicotinate, nicotinamide, trigonelline, allantoin, adenine, guanine, cytosine, thymine, uracil, and n-hexadecane. The traits present in only some of the strains are shown in Table 1.

DISCUSSION

The genus *Alteromonas* as currently defined (Baumann, Gauthier, and Baumann, in press) consists of gram-negative,

* Corresponding author.

FIG. 1. Electron micrograph of *A. nigrifaciens* 217^T in the exponential phase of growth in liquid medium. $\times 24,000$. Bar = 0.5 μm .

polarly flagellated, rod-shaped organisms which do not accumulate poly- β -hydroxybutyrate as an intracellular reserve product. These bacteria are chemoorganotrophs that are capable of respiratory but not fermentative metabolism, with molecular oxygen as the universal electron acceptor. None denitrifies or has a constitutive arginine dihydrolase system. All require Na^+ or a seawater base for growth. The G+C contents of the DNAs range from 38 to 50 mol%. "*P. nigrifaciens*" strain 217^T (= ATCC 19375^T) has all of these properties and is therefore assigned to the genus *Alteromonas*. The following selected phenotypic properties distinguish strain 217^T from all of the previously described species of *Alteromonas* by 6 to 11 traits: production of a brown or black pigment; ability to utilize D-fructose, maltose, succinate, fumarate, citrate, and glycerol; and inability to produce an extracellular amylase or utilize D-mannose, sucrose, N-acetylglucosamine, erythritol, and D-sorbitol. These findings, together with the immunological studies of the iron-containing superoxide dismutases and glutamine synthetases of *Alteromonas* (DeLong et al., in press), indicate that strain 217^T constitutes a distinct species, which we designate *A. nigrifaciens* sp. nov., nom. rev.

Below is a description of *A. nigrifaciens*, as well as descriptions of *A. macleodii* and *A. haloplanktis*. In our previous publications (1, 3, 5) we did not include the

phenotypic properties of the type strain of *A. macleodii* or the properties of the remaining strains of the latter two species.

Description of *Alteromonas nigrifaciens*. *Alteromonas nigrifaciens* sp. nov., nom. rev. (ni.gri.fa'ci.ens. L. adj. *niger* black; L. v. *facio* to make; M.L. part. adj. *nigrofaciens* blackening) cells are straight rods 0.8 to 1.2 μm wide and 1.8 to 2.3 μm long and are motile by single flagella at one pole (Fig. 1). Grows at 4 to 30°C but not at 35°C. Produces a brown or black pigment which is especially prominent at lower temperatures (Fig. 2). Produces an extracellular gelatinase and lipase but not an amylase, alginase, or chitinase. Utilizes 26 organic compounds, including hexoses, disaccharides, fatty acids, tricarboxylic acid cycle intermediates, alcohols, and amino acids. The G+C content of the DNA is 42.9 mol%.

The type strain is strain 217 (= ATCC 19375).

Description of *A. macleodii*. Straight rods 0.9 to 1.3 μm wide and 1.8 to 2.5 μm long that are motile by single flagella at one pole. Produces an extracellular amylase, gelatinase, and lipase but not a chitinase. Utilizes 26 to 44 organic compounds, including hexoses, disaccharides, sugar acids, and amino acids. The G+C content of the DNA is 44.9 to 46.4 mol%.

The type strain is strain 107 (= ATCC 27126).

Description of *A. haloplanktis*. Straight rods 0.8 to 1.2 μm wide and 1.9 to 3.0 μm long that are motile by single flagella at one pole. Produces an extracellular gelatinase and lipase but not an alginase. Utilizes 20 to 38 organic compounds, including hexoses, disaccharides, fatty acids, tricarboxylic

FIG. 2. Colony morphology and pigmentation of *A. nigrifaciens* grown on complex medium for 2 days at 30°C (a) and for 3 days at 4°C (b). Bar = 2 mm.

TABLE 1. Phenotypic properties not universally present in *A. nigrifaciens*, *A. macleodii*, and *A. haloplanktis*^a

Property	Phenotype of <i>A. nigrifaciens</i> strain 217 ^T (= ATCC 19375 ^T)	<i>A. macleodii</i>				<i>A. haloplanktis</i>			
		No. of strains positive (<i>n</i> = 21)	Phenotype of strain 107 ^T (= ATCC 27126 ^T)	Less common pheno- type	Strain(s) with less common phenotype	No. of strains positive (<i>n</i> = 23)	Phenotype of strain 215 ^T (= ATCC 14393 ^T)	Less common pheno- type	Strain(s) with less common phenotype
NO ₃ ⁻ → NO ₂ ⁻	-	2	-	+	103, 106	9	-	+	119-121, 124, 126, 131, 318-320
Amino acid re- quirement	+	0	-			7	-	+	214, 318-323
Growth at:									
4°C	+	0	-			2	-	+	214, 216
35°C	-	21 ^b	+			21 ^b	-	-	214, 215 ^T
40°C	-	15	+	-	98, 99, 103- 105, 118	0	-		
Production of:									
Amylase	-	19 ^b	+	-	106, 118	3	-	+	121, 322, 323
Gelatinase	+	20 ^b	+	-	106	23 ^b	+		
Alginase	-	3	+	+	98, 99, 107 ^T	0	-		
Chitinase	-	0	-			14	-	-	214, 215 ^T , 216, 318-323
Utilization of:									
D-Xylose	-	4	+	+	98, 107 ^T , 116, 117	1	-	+	122
L-Arabinose	-	0	-			15	-	-	119, 121, 126, 214, 215 ^T , 216, 322, 323
L-Rhamnose	-	1	-	+	116	2	-	+	122, 125
D-Mannose	-	0	-			21 ^b	-	-	215 ^T , 216
D-Galactose	+	21 ^b	+			5	-	+	120, 121, 127, 128, 214
D-Fructose	+	21 ^b	+			15	+	-	119-121, 124, 126, 216, 322, 323
Sucrose	-	21 ^b	+			21 ^b	-	-	214, 215 ^T
Trehalose	-	19 ^b	+	-	109, 110	19 ^b	-	-	120, 214, 215 ^T , 216
Cellobiose	-	21 ^b	+			0	-		
Melibiose	+	20 ^b	+	-	109	0	-		
Lactose	+	21 ^b	+			2	-	+	127, 128
Salicin	-	21 ^b	+			0	-		
Gluconate	+	18 ^b	+	-	102, 106, 108	1	-	+	214
Galacturonate	-	18 ^b	+	-	102, 103, 118	1	-	+	130
N-acetylglu- cosamine	-	7	+	+	98, 99, 101, 107 ^T , 108, 113, 116	21 ^b	-	-	215 ^T , 216
Butyrate	+	21 ^b	+			12	-	-	121, 123-125, 127-132, 215 ^T
Isobutyrate	+	21 ^b	+			20 ^b	-	-	121, 215 ^T , 216
Valerate	+	21 ^b	+			17	+	-	123-126, 130, 132
Isovalerate	-	5	-	+	104-106, 109, 114	1	-	+	214
Caproate	+	19 ^b	+	-	113, 117	2	-	+	214, 216
Heptanoate	-	11	+	-	109-118	7	-	+	126, 129-132, 214, 216
Caprylate	-	21 ^b	+			18	+	-	121, 127, 128, 322, 323
Pelargonate	-	21 ^b	+			19 ^b	+	-	121, 214, 322, 323
Caprate	-	21 ^b	+			23 ^b	+		
Succinate	+	0	-			23 ^b	+		
Fumarate	+	0	-			23 ^b	+		
DL-β-Hydroxy- butyrate	-	12	-	-	98-104, 107 ^T , 108	13	-	-	120, 123-126, 130-132, 215 ^T , 216
DL-Lactate	+	16	+	-	100-103, 106	2	-	+	122, 125
DL-Glycerate	-	21 ^b	+			0	-		
Citrate	+	0	-			21 ^b	-	-	215 ^T , 216
Aconitate	+	0	-			21 ^b	-	-	215 ^T , 216

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TABLE 1—Continued

Property	Phenotype of <i>A. nigrifaciens</i> strain 217 ^T (= ATCC 19375 ^T)	<i>A. macleodii</i>				<i>A. haloplanktis</i>			
		No. of strains positive (n = 21)	Phenotype of strain 107 ^T (= ATCC 27126 ^T)	Less common phenotype	Strain(s) with less common phenotype	No. of strains positive (n = 23)	Phenotype of strain 215 ^T (= ATCC 14393 ^T)	Less common phenotype	Strain(s) with less common phenotype
Mannitol	—	9	+	+	98, 99, 107 ^T , 108, 111–113, 115, 116	11	—	+	122–124, 127, 128, 131, 132, 318–321
Glycerol	+	21 ^b	+			0	—		
Propylene glycol	—	5	—	+	109, 110, 114–116	3	—	+	119–121
Ethanol	+	17 ^b	+	—	100, 101, 103, 118	18	—	—	215 ^T , 318, 319, 322, 323
n-Propanol	+	15	+	—	100, 101, 103, 112, 113, 118	15	—	—	122, 131, 132, 215 ^T , 318, 319, 322, 323
Glycine	—	14	+	—	101, 102, 104, 108, 111, 112, 115	14	+	—	119–123, 132, 216, 322, 323
L-α-Alanine	+	19 ^b	+	—	104, 110	23 ^b	+		
L-Serine	+	9	—	+	105, 109–112, 114–117	23 ^b	+		
L-Threonine	—	0	—	—		7	—	+	214, 318–323
L-Leucine	—	15	—	—	101–104, 107 ^T , 108	12	+	—	119–128, 132
L-Isoleucine	—	13	—	—	99, 102–108	2	—	+	319, 321
L-Valine	—	11	—	—	98, 99, 102–106, 107 ^T , 108, 117	4	—	+	130, 319, 321, 322
L-Aspartate	—	9	—	+	109, 111–118	11	—	+	119, 121–124, 127–132
L-Glutamate	+	15	—	—	102–104, 106, 107 ^T , 108	14	+	—	120, 122, 216, 318–323
L-Lysine	—	1	—	+	99	0	—		
L-Arginine	—	8	—	+	109–116	13	—	—	119–124, 215 ^T , 216, 318, 319
L-Ornithine	—	0	—			1	—	+	214
L-Citrulline	—	0	—			2	+	+	214, 215 ^T
L-Histidine	—	0	—			11	—	+	119, 126–132, 318, 319, 323
L-Proline	—	1	—	+	101	12	+	—	119–125, 216, 318, 319, 321
L-Tyrosine	+	18 ^b	—	—	102, 107, 117	21 ^b	+	—	122, 216
L-Phenylalanine	—	0	—			1	—	+	214
Hippurate	—	0	—			1	—	+	214

^a Strain 217^T was the only *A. nigrifaciens* strain tested. The 21 strains of *A. macleodii* tested were strains 98, 99, 100, 101, 102, 103, 104, 105, 106, 107^T, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, and 118. The 23 *A. haloplanktis* strains tested were strains 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 214, 215^T, 216, 318, 319, 320, 321, 322, and 323.

^b More than 80% of the strains positive.

acid cycle intermediates, and amino acids. The G+C content of the DNA is 41.8 to 44.4 mol%.

The type strain is strain 215 (= ATCC 14393).

The properties which distinguish *A. macleodii* and *A. haloplanktis* from the remaining species of *Alteromonas* are given in *Bergey's Manual of Systematic Bacteriology*, 9th ed. (Baumann, Gauthier, and Baumann, in press). Isolates of the marine species *Vibrio nigrifaciens* (P. Baumann, A. L. Furniss, and J. V. Lee, in *Bergey's Manual of Systematic Bacteriology*, 9th ed., in press) and group I-2 (1) produce a blueish black pigment or a brownish black pigment. *V. nigrifaciens* is differentiated from *A. nigrifaciens* by its ability to ferment carbohydrates. Members of group I-2 are obligately respiratory organisms which are differentiated from *A. nigrifaciens* by their inability to utilize carbohydrates and their G+C contents (61 to 64 mol%). A requirement for approximately 250 mM NaCl for optimal growth

distinguishes *A. nigrifaciens* from most common gram-negative terrestrial microorganisms.

ACKNOWLEDGMENTS

This study was supported by grant DEB-8205938 from the National Science Foundation, as well as by the Aquaculture Program, University of California Agricultural Experiment Station.

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