CURRENT MICROBIOLOGY, Vol. 3 (1980), pp. 311–315

Current Microbiology
An International Journal

Characterization of *Alteromonas hanedai* (sp. nov.), a Nonfermentative Luminous Species of Marine Origin

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Abstract. Eleven marine luminous isolates, which could not be identified with previously studied species of luminous marine bacteria, were subjected to an extensive characterization. The results indicated that these strains were phenotypically similar, had a G+C content in their DNA of 45 mol%, and differed from all previously characterized luminous species by their inability to ferment sugars. On the basis of these and other properties, the 11 luminous strains were assigned to the genus *Alteromonas* and given the species designation *A. hanedai*. Strain 281 (ATCC 33224) has been designated as the type strain of this new species.

Bacteria able to emit light are common in the marine environment [5,6,13,14]. This property has been found in the marine species *Beneckea harveyi, B. splendida* biotype I, *Photobacterium fischeri, P. logei, P. phosphoreum,* and *P. leiognathi* [2,4,18,19,20]. A simple set of diagnostic traits has been devised for the identification of these species [2,17] which has been recently applied in a number of ecological studies [15,20,21,22]. An additional property, useful in distinguishing species of *Photobacterium* from *Beneckea,* is the decay kinetics of light emission by luciferase. Species of the former genus have "fast" decay kinetics while those of the latter genus have "slow" decay kinetics [13,14]. During a routine screening of the numerous marine luminous isolates conducted over the past several years, two of us (K.H.N., B.M.T.) found a number of strains having luciferase kinetics similar to that of species of *Photobacterium* which, however, on the basis of a limited set of diagnostic traits could not be accommodated into any of the previously described marine luminous species. The present report is a characterization of these strains which are shown to constitute a new species differing from other species of luminous bacteria by the inability to ferment sugars.

Materials and Methods

The 11 strains characterized in this study were isolated from the following sources: strain 281 (Arctic sediments), 282 and 283 (seawater in the Antarctic), strains 284–291 (Sannich Inlet, British Columbia, Canada, from depths of 100–220 m). The methods used for the phenotypic characterization of strains have been described in detail [4,6,18]. Since all strains required organic growth factors, the nutritional spectrum was determined in media supplemented with 0.05 g/liter yeast extract and 0.05 g/liter tryptone and examined after 3, 5, and 7 days of incubation at 15°C. Tests for chitinase production were incubated for 14 days, after which time the colonies were gently washed off to better detect the zones of clearing. Samples were prepared for electron microscopy as previously described [1]. The mol% G+C content of DNA was determined in duplicate by CsCl density gradient centrifugation [15].

Results and Discussion

Morphology. All of the 11 strains were motile straight rods (Fig. 1) which when stained for flagella by the Liefson method [6] were found to have a single polar flagellum (Fig. 2). An electron microscopic examination of three isolates indicated that the polar flagellum was not surrounded by a sheath (Fig. 3) of the type found in species of *Beneckea* [1]. None of the strains accumulated poly-β-hydroxybutyrate as an intracellular reserve product.

General physiological properties. All strains were oxidase positive and reduced nitrates to nitrites but not to nitrogen gas. None were able to grow on a minimal medium containing 0.1% potassium acetate, 0.1% L-glutamate, and 0.1% L-alanine. If this medium was supplemented with vitamin-free casein hydrolysate and 50 mg/liter of L-tryptophan, L-methionine, and L-cysteine, 5 of the 11 strains were able to

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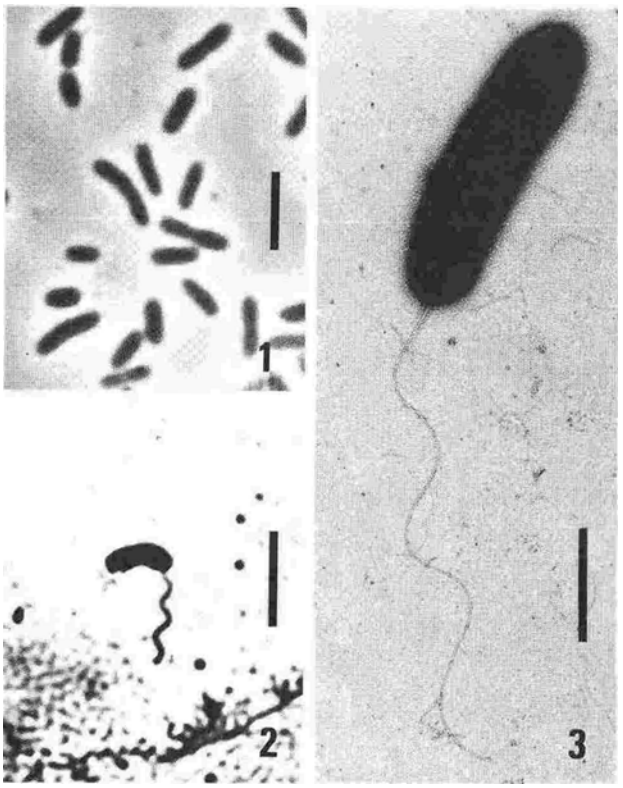

Fig. 1. Phase-contrast micrographs of *Alteromonas hanedai* strain 281 in exponential phase of growth. Marker represents 5 μm. Fig. 2. Liefson flagella stain of strain 281. Marker represents 5 μm. Fig. 3. Electron micrograph of strain 281, negatively stained. Marker represents 1 μm.

grow. All of the strains grew in the minimal medium supplemented with 0.1% yeast extract, while none grew in this medium if the sodium ion in the seawater base was replaced by equimolar amounts of potassium. All strains grew at 4 and 20°C, none grew at 30°C.

The ability to ferment D-glucose, D-galactose, and *N*-acetylglucosamine was tested in two media (F-1 and F-2) which have been previously described [4,6]. In F-1 medium (which was sealed by means of an agar plug) no visible growth was apparent, the pH after 7 days' incubation being either unchanged or decreased by up to 0.25 pH units. In F-2 medium (which contained thioglycolate but was not sealed) growth was visible only on the surface but not in the anaerobic portion. The pH after 7 days' incubation was unchanged or increased by up to 0.3 pH units. Three strains were also tested for growth in F-2 medium incubated under an atmosphere of 90% N_2 and 10% CO_2 with no visible growth. When tested under similar conditions species of *Beneckea* and *Photobacterium* grew well throughout the medium and lowered the pH from 7.5 to a value below 5.5.

Table 1. Nutritional properties of *Alteromonas hanedai*.[a]

Traits	Phenotype of Type strain ATCC 33224	Phenotype of Species (11 strains)
Amylase	−	−
Lipase	+	+
Gelatinase	+	+
Chitinase	+	+
Alginase	−	−
D-Ribose	+	9
D-Glucose	+	9
D-Galactose	+	9
D-Gluconate	−	2
N-Acetylglucosamine	+	+
Acetate	+	+
Propionate	+	+
Butyrate	+	+
Caproate	+	3
Heptanoate	+	3
Caprylate	+	1
Pelargonate	+	3
Caprate	+	+
DL-Glycerate	+	+
Citrate	−	1
Pyruvate	+	+
Glycine	+	+
L-α-Alanine	+	+
D-α-Alanine	−	2
L-Serine	+	+
L-Threonine	+	+
L-Leucine	+	3
L-Isoleucine	+	+
L-Aspartate	+	+
L-Glutamate	+	+
L-Tyrosine	−	8
Putrescine	+	+
Spermine	+	+

[a] +, All strains positive; −, all strains negative; numbers indicate number of positive strains. The following compounds did not support growth: D-xylose, L-arabinose, L-rhamnose, D-mannose, D-fructose, sucrose, trehalose, maltose, cellobiose, melibiose, lactose, salicin, glucuronate, galacturonate, isobutyrate, valerate, isovalerate, malonate, succinate, fumarate, glutarate, DL-malate, L-(+)-tartrate, DL-β-hydroxybutyrate, DL-lactate, aconitate, erythritol, mannitol, *m*-inositol, adonitol, glycerol, propylene glycol, ethanol, *n*-propanol, benzoate, *p*-hydroxybenzoate, phenylacetate, quinate, β-alanine, L-valine, L-lysine, L-arginine, L-ornithine, L-citrulline, γ-aminobutyrate, δ-aminovalerate, L-histidine, L-proline, L-phenylalanine, betaine, sarcosine, hippurate.

Mol% G+C. The G+C content of the DNAs from strains 281, 282, 283, 284, and 287 was 46.4, 45.4,

Table 2. Properties distinguishing *Alteromonas hanedai* from other species of *Alteromonas*.[a]

Traits	*A. hanedai* (11)[b]	*A. macleodii* (21)	*A. haloplanktis* (25)	*A. espejiana* (18)	*A. undina* (8)	*A. rubra* (3)	*A. luteoviolaceus* (16)	*A. citrea* (3)	*A. aurantia* (4)	*A. communis* (33)	*A. vaga* (17)
Mol% G+C content of DNA	45	46	43	43	43	47	42	42	41	47	48
Straight rods[c]	+	+	+	+	+	+	+	+	+	−	+
Ring cleavage[d]	−	−	−	−	−	−	ND[e]	−	−	*m*	*m*
Oxidase	+	+	+	+	+	+	+	+	+	+	−
Luminescence	+	−	−	−	−	−	−	−	−	−	−
Growth at 4°C	+	−	2	−	**4**	−	ND	−	+	−	−
Growth at 35°C	−	+	**21**	8	−	+	+	1	−	+	+
Growth at 40°C	−	15	−	−	−	−	−	1	−	+	−
Organic growth factors required	+	−	6	+	+	+	ND	+	+	−	−
Amylase	−	**19**	4	+	5	+	15	+	+	−	−
Gelatinase	+	**20**	+	+	+	+	+	+	+	−	−
Lipase	+	+	+	+	+	+	+	+	+	−	−
Alginase	−	3	−	+	−	ND	ND	ND	−	−	−
Chitinase	+	−	16	−	+	−	−	−	−	−	−
D-Mannose	−	−	**21**	12	−	+	−	+	+	**29**	+
D-Galactose	**9**	+	5	+	−	−	ND	−	−	11	**15**
Sucrose	−	+	**23**	+	+	−	−	−	−	−	1
Cellobiose	−	+	−	9	−	−	−	−	−	−	14
Melibiose	−	**20**	−	+	−	ND	ND	ND	−	−	−
Lactose	−	+	1	+	−	−	−	−	−	−	−
Salicin	−	+	−	−	−	−	ND	−	−	−	−
D-Gluconate	2	**18**	1	−	−	−	ND	−	−	+	+
N-Acetylglucosamine	+	7	**23**	−	+	ND	ND	ND	+	−	**15**
Succinate	−	−	+	−	+	−	ND	−	−	+	+
Fumarate	−	−	+	−	+	−	ND	−	−	+	+
DL-Lactate	−	16	2	−	−	−	ND	−	−	+	**16**
DL-Glycerate	+	+	−	−	−	ND	ND	ND	−	4	−
Citrate	**1**	−	**21**	**17**	−	−	ND	−	−	+	+
Aconitate	−	−	**21**	**17**	−	ND	ND	ND	−	+	**16**
Erythritol	−	−	−	−	−	−	ND	−	−	−	+
Mannitol	−	9	11	+	−	−	−	−	−	+	+
Glycerol	−	+	−	−	−	−	−	−	−	+	+
γ-Aminobutyrate	−	−	−	−	−	ND	ND	ND	−	+	**16**
L-Tyrosine	8	**18**	**23**	+	+	ND	ND	ND	+	−	−
Putrescine	+	−	−	−	−	ND	ND	ND	ND	+	**15**
Sarcosine	+	−	−	−	−	ND	ND	ND	−	+	+
Sorbitol, DL-malate, α-ketoglutarate, *m*-hydroxybenzoate	−	−	−	−	−	−	ND	−	−	+	+
Pigmentation	−[f]	−	−	−	−	+[g]	+[h]	+[i]	+[j]	−	−

[a] +, All strains positive; −, all strains negative; numbers indicate number of positive strains; numbers in boldface indicate that the number represents 80% or more of the strains. Data from this study and references [3,6,7,9,10,11,12,17].

[b] Number in parentheses refers to number of strains studied.

[c] +, Straight rod; −, curved rods.

[d] Mechanism of aromatic ring cleavage by species capable of growth on aromatic compounds.

[e] Not determined.

[f] Some strains upon initial isolation produce a diffusable brown pigment on complex medium.

[g] Prodigiosin.

[h] Violacein.

[i] Lemon-yellow, noncarotenoid pigment.

[j] Orange, noncarotenoid pigment.

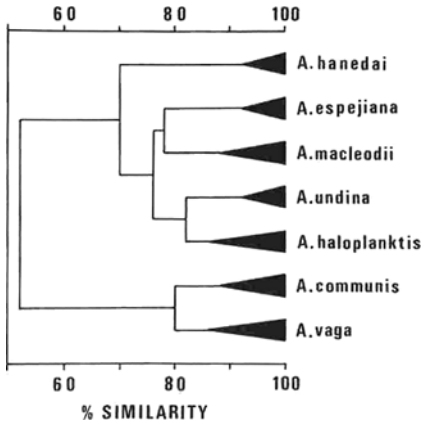

Fig. 4. Numerical analysis of phenotypic properties of species of *Alteromonas*.

44.9, 44.9, and 44.4 mol%, respectively, with a mean value of 45.2 ± 0.8 mol%.

Taxonomic assignments and nutritional analysis. The morphological properties, mol% G+C contents of the DNAs, as well as the requirement of sodium ion for growth indicate that the 11 luminous isolates have properties of the previously described genus *Alteromonas* [3,6]. The results of a nutritional screening of these strains indicated considerable phenotypic similarity (Table 1). When these data along with those from six previously characterized species of *Alteromonas* [3,7,17] were subjected to numerical analysis [4], the results presented in Fig. 4 were obtained. The luminous isolates were phenotypically similar and distinct from other species of *Alteromonas*, justifying a new species designation. We propose to name this species *Alteromonas hanedai* in honor of Yata Haneda, a Japanese biologist who has made numerous contributions to our knowledge of the biology of bioluminescence. Strain 281 has been designated as the type strain and deposited in the American Type Culture Collection (ATCC 33224).

Table 2 gives the diagnostic properties differentiating *A. hanedai* from other species of *Alteromonas*. Since there is considerable interest in the ecology of the marine luminous bacteria, it is also useful to differentiate *A. hanedai* from other luminous species on the basis of traits other than fermentation. For this purpose, we have compiled Table 3. Since several of the luminous bacteria prefer lower temperatures, it is advisable that in ecological studies the initial incubation be conducted at 15°C. The nutritional screening can also be performed at this temperature with most of the tests scored after 3, 5, and 7 days of incubation.

Table 3. Some distinguishing properties for identifying luminous species of *Alteromonas*, *Photobacterium*, and *Beneckea*.[a]

Traits	*A. hanedai* (11)[b]	*P. phosphoreum* (79)	*P. leiognathi* (30)	*P. fischeri* (12)	*P. logei* (11)	*B. harveyi* (85)	*B. splendida* biotype 1 (4)
Mol% G+C content in DNA	45	42	43	40	40	46	46
Peritrichous[c]	−	−	−	−	−	**73**	−
Sheathed polar flagella[d]	−	−	−	+	+	+	+
Number of polar flagella[d]	1	1–3	1–3	2–8	2–8	1	1
Curved rods[e]	−	−	1	1	1	−	+
Yellow-orange pigment	−	−	−	+	+	−	−
Poly-β-hydroxybutyrate accumulation	−	+	+	−	−	−	−
Arginine dihydrolase	−	−	−	−	−	−	+
Luminescence	+	+	+	+	**10**	61	+
Fermentation of D-glucose	−	+	+	+	+	+	+
Gas from D-glucose fermentation	−	**71**	2	−	−	−	−
Production of acetoin and/or diacetyl	−	**71**	15	−	−	−	−
Growth at 4°C	+	**75**	−	−	+	−	1
Growth at 30°C	−	**67**	+	**11**	−	+	+
Growth at 35°C	−	−	**28**	8	−	+	3
Amylase	−	−	−	−[f]	−	+	+
Lipase	+	−	**24**	**11**	**9**	+	+
Gelatinase	+	−	−[g]	1	−	**84**	+
Maltose	−	**78**	−	**11**	+	+	+
Cellobiose	−	−	−	**11**	+	+	+
D-Gluconate	2	**68**	+	−[f]	**10**	**84**	1
D-Glucuronate	−	41	−	−	−	+	+
Acetate	+	−	**25**	−	−	**79**	3
Propionate	+	−	−	−	−	+	+
DL-Lactate	−	13	+	−	−	+	+
α-Ketoglutarate	−	−	1	−	−	**84**	+
Mannitol	−	−	−	**11**	**10**	+	+
D-α-Alanine	2	−	−	−[f]	−	**84**	+
L-Tyrosine	8	−	−	−	−	+	+
Putrescine	+	−	−	−	−	−	−
Spermine	+	−	−	−	−	−	−

[a] +, All strains positive; −, all strains negative; numbers indicate number of positive strains; boldface numbers indicate that the number represents 80% or more of the strains. Data from this study and references [2,6,18,19,20].

[b] Number in parentheses refers to number of strains studied.

[c] Strains having unsheathed peritrichous flagella in addition to a sheathed polar flagellum when grown on solid medium.

[d] Determined in liquid medium.

[e] +, All curved rods; −, all straight rods; numbers indicate number of curved rods.

[f] A few strains of *P. fischeri* have been found to be positive for this trait [8,22].

[g] Some strains of *P. leiognathi* have been found to be positive for this trait [21].

ACKNOWLEDGMENTS

We are grateful to Sookie S. Bang for the phenotypic characterization of one of the isolates. This investigation was supported by NIH research grant number AI 15924 from the Institute of Allergy and Infectious Diseases.

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