DNA Relatedness among Nonpigmented Species of *Alteromonas* and Synonymy of *Alteromonas haloplanktis* (ZoBell and Upham 1944) Reichelt and Baumann 1973 and *Alteromonas tetraodonis* Simidu et al. 1990

MASAYO AKAGAWA-MATSUSHITA,^{1*} YOSUKE KOGA,¹ AND KAZUhide YAMASATO²

Department of Chemistry, University of Occupational and Environmental Health, Yahatanishi-ku, Kitakyushu 807,¹ and Institute of Applied Microbiology, The University of Tokyo, Bunkyo-ku, Tokyo 113,² Japan

The taxonomic relationships among nonpigmented species of the genus *Alteromonas* were assessed by DNA-DNA hybridization. The species formed two groups based on DNA homology values and moles percent G+C of DNA: *Alteromonas macleodii*, the type species, had a moles percent G+C content 4 to 7% higher than those of the other group, and it had less than 5% DNA homology to the second group formed by the remaining species. The remaining species formed seven DNA homology subgroups, of which five corresponded to the type strains of *A. atlantica*, *A. espejiana*, *A. carrageenovora*, *A. undina*, and *A. nigrifaciens*. A sixth subgroup included the type strains of *A. haloplanktis* and *A. tetraodonis*, which showed hybridizations of more than 80%. The species in the second group were related to each other at the genus level, but a nomenclatural proposal is postponed. The hybridization data showed that the name *A. tetraodonis* Simidu et al. 1990 should be recognized as a junior subjective synonym of *A. haloplanktis* (ZoBell and Upham 1944) Reichelt and Baumann 1973. The seventh subgroup included two as yet unassigned strains which were misidentified as to species.

The genus *Alteromonas* originally consisted of three species of gram-negative, aerobic, nonpigmented, polarly flagellated marine bacteria which resembled *Pseudomonas* but had a lower moles percent G+C content (38 to 50%) (5). A

further seven nonpigmented and five pigmented species have since been described as members of the genus. The phylogenetic analysis by rRNA-DNA hybridization reported by Van Landschoot and De Ley (22) showed that the genus

TABLE 1. Strains used^a

Species	Strain	Source	Other designation(s)	Previous assignment ^b	Reference(s)
<i>Alteromonas macleodii</i>	IAM 12920 ^T	ATCC 27126	NCIMB 1963, Baumann 107		5, 10, 22
<i>Alteromonas atlantica</i>	IAM 12927 ^T	NCIMB 301	ATCC 19262, IAM 12376, CIP 63.28, JCM 8845	" <i>Pseudomonas atlantica</i> "	2, 22, 23
<i>Alteromonas carrageenovora</i>	IAM 12662 ^T	IFO 12985	ATCC 43555, NCIMB 302, CIP 103674, JCM 8851	" <i>Pseudomonas carrageenovora</i> "	2, 11, 22
<i>Alteromonas espejiana</i>	IAM 12640 ^T	ATCC 29659	NCIMB 2127, Baumann 261		9, 22
<i>Alteromonas haloplanktis</i>	IAM 12915 ^T	ATCC 14393	NCIMB 2084, ZoBell & Upham 545, Baumann et al. 215	" <i>Vibrio haloplanktis</i> "	17, 22
<i>Alteromonas haloplanktis</i>	IAM 12916	ATCC 19648	Johnson ASU-1	" <i>Vibrio marinopraescens</i> "	22
<i>Alteromonas haloplanktis</i>	IAM 12917	ATCC 23821	NCIMB 1545, Colwell 651	" <i>Pseudomonas enalia</i> "	17, 22
<i>Alteromonas haloplanktis</i>	IAM 12918	ATCC 27127	NCIMB 1964, Baumann 121	" <i>Alteromonas marinopraescens</i> "	17, 22
<i>Alteromonas haloplanktis</i>	IAM 12919	NCIMB 19	ATCC 19855, MacLeod B-16	<i>Pseudomonas</i> sp.	7
<i>Alteromonas undina</i>	IAM 12922 ^T	ATCC 29660	NCIMB 2128, Baumann 272		9, 22
<i>Alteromonas undina</i>	NS-110	M. Maeda ^c			
<i>Alteromonas nigrifaciens</i>	IAM 13010 ^T	ATCC 19375	NCIMB 8614, NCTC 10691, NCDO 755	" <i>Pseudomonas nigrifaciens</i> "	6, 8, 10, 22
<i>Alteromonas nigrifaciens</i>	IAM 13161	ATCC 25013			6, 11, 22
<i>Alteromonas tetraodonis</i>	IAM 14160 ^T	U. Simidu ^d			
<i>Marinomonas communis</i>	IAM 12914 ^T	ATCC 27118	NCIMB 1961, Baumann 8	<i>Alteromonas communis</i>	5, 8, 22
<i>Marinomonas vaga</i>	IAM 12923 ^T	ATCC 27119	NCIMB 1962, Baumann 40	<i>Alteromonas vaga</i>	5, 8, 22
<i>Escherichia coli</i>	IAM 1264	ATCC 10798			

^a Abbreviations: ^T, type strain; ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection des Bacteries de l'Institut Pasteur, Paris, France; JCM, Japan Collection of Microorganisms, RIKEN, Wako-shi, Saitama, Japan; IAM, Institute of Molecular and Cellular Biosciences (formerly Institute of Applied Microbiology), The University of Tokyo, Bunkyo-ku, Tokyo, Japan; IFO, Institute for Fermentation Osaka, Osaka, Japan; NCDO, National Collection of Dairy Organisms, Reading, England; NCIMB, National Collections of Industrial and Marine Bacteria, NCIMB Ltd., Torry Research Station, Aberdeen, Scotland; NCTC, National Collection of Type Cultures, London, England.

^b Names in quotation marks have not been validated.

^c National Research Institute of Aquaculture, Nansei, Mie, Japan.

^d Ocean Research Institute, The University of Tokyo, Nakano-ku, Tokyo, Japan.

* Corresponding author.

TABLE 2. DNA relatedness among *Alteromonas* strains

Strain	G+C content (mol%)	% Reassociation with ³ H-labeled DNA from ^a :								
		<i>A. macleodii</i> IAM 12920 ^T	<i>A. atlantica</i> IAM 12927 ^T	<i>A. carrageenovora</i> IAM 12662 ^T	<i>A. espejiana</i> IAM 12640 ^T	<i>A. haloplanktis</i> IAM 12915 ^T	<i>A. tetraodonis</i> IAM 14160 ^T	<i>A. undina</i> IAM 12922 ^T	<i>A. undina</i> NS-110	<i>A. nigrifaciens</i> IAM 13010 ^T
<i>A. macleodii</i> IAM 12920 ^T	46.2 ^b	100	6 ^b	4 ^b	7 ^b	5	6	8	7	5
<i>A. atlantica</i> IAM 12927 ^T	41.2 ^b	5	100	26 ^b	40 ^b	30	23 ^b	19	18	28
<i>A. carrageenovora</i> IAM 12662 ^T	39.5 ^b	4	21 ^b	100	43 ^b	21	26 ^b	27	32	24
<i>A. espejiana</i> IAM 12640 ^T	41.4 ^b	5	43 ^b	45 ^b	100	41	22 ^b	17		20
<i>A. haloplanktis</i> IAM 12915 ^T	41.6 ^b	2	27 ^b	21 ^b	23 ^b	100	84	57	42	35
<i>A. haloplanktis</i> IAM 12916	40.5					80	81	51	39	
<i>A. haloplanktis</i> IAM 12917	40.9					87	109	61	62	
<i>A. tetraodonis</i> IAM 14160 ^T	40.8 ^b		30	27	19	82	100	50	48	36
<i>A. undina</i> IAM 12922 ^T	40.1 ^b		25 ^b	21 ^b	25 ^b	47	52	100	100	22
<i>A. undina</i> NS-110	40.3		25	21	36	59	57	97	100	
<i>A. nigrifaciens</i> IAM 13010 ^T	40.6 ^b	5	32 ^b	23 ^b	21 ^b	35	30	32	31	100
<i>A. haloplanktis</i> IAM 12919	41.1	5	39	40	27	33		23		67
<i>A. haloplanktis</i> IAM 12918	42.2	4	19	14	11	16		13		12
<i>A. nigrifaciens</i> IAM 13161						22	29	23		31
<i>M. communis</i> IAM 12914 ^T	47.0 ^b	4	4 ^b	4 ^b	5 ^b	4	3	5	3	3
<i>M. vaga</i> IAM 12923 ^T	48.4 ^b	3	4 ^b	4 ^b	4 ^b	4	3	3	3	4
<i>E. coli</i> IAM 1264	51.3 ^b	5	4 ^b	3 ^b	3 ^b	3	4 ^b	4	7	5

^a Reassociation values are averages of four independent determinations.^b Data from reference 2.

Alteromonas consisted of two rRNA groups which were so remotely related as to warrant separate families (11). They also showed that several *Pseudomonas* species should be classified as members of the genus *Alteromonas* but left them unassigned. *Alteromonas* species and *Alteromonas*-like bacteria are widely distributed and frequently isolated from marine environments. They are ecologically and economically important microorganisms (3, 4, 12–16, 18, 21).

The identification of *Alteromonas*-like isolates is difficult because of great intraspecific variation in their characters. Most species were proposed on a biochemical and nutritional basis combined with taxometric clustering (6, 7, 9, 12). They were described mostly without genetic evidence, except for two recently described species, *Alteromonas atlantica* and *A. carrageenovora* (2). The proposal of *A. tetraodonis* was supported by partial sequencing of 16S rRNA (19). The nonpigmented species of *Alteromonas* are physiologically similar (2), and the identification and classification of such strains need genetic analysis.

In this study DNA-DNA hybridization experiments were performed to confirm the validity of the existing species and to clarify the taxonomic structure of the genus *Alteromonas*, with particular attention to nonpigmented species.

MATERIALS AND METHODS

Bacterial strains. The strains of *Alteromonas* that we used are listed in Table 1. Two type strains of *Marinomonas* species and *Escherichia coli* IAM 1264 (strain K-12) were included for reference. All strains were maintained on slopes of Marine Agar 2216 (Difco Laboratories, Detroit, Mich.) at 25°C and transferred every month. For long-term preservation, cell suspensions in Marine Broth 2216 (Difco) supplemented with 10% glycerol were stored in liquid nitrogen.

Determination of DNA base composition and DNA-DNA hybridization. Methods for DNA preparation were described previously (1). The determination of the moles percent G+C content of DNA involved high-performance liquid chroma-

tography as described by Tamaoka and Komagata (20), using a DNA-GC kit (Yamasa shoyu, Choshi, Japan). DNA-DNA hybridization was carried out by the membrane filter method under nonstringent conditions (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] plus 0.1% sodium dodecyl sulfate, 62°C) by a procedure we have employed previously (2).

RESULTS AND DISCUSSION

The moles percent G+C content of DNAs and percent homology values for all of the strains we studied are presented in Table 2. The distinctness of the G+C moles percent values and the very low DNA homology values between *A. macleodii*, the type species, and the other species of the genus reflect the phylogenetic unrelatedness that has been shown by rRNA-DNA hybridizations (22). The nonpigmented *Alteromonas* species were thus divided into two obvious groups. Table 2 shows seven subgroups apart from the type species; five of these included the type strains of a species (*A. atlantica*, *A. carrageenovora*, *A. espejiana*, *A. undina*, and *A. nigrifaciens*). One of the subgroups included the type strains of both *A. haloplanktis* and *A. tetraodonis*, which showed 82 to 84% hybridization. It showed <5% homology with either *Marinomonas communis* or *Marinomonas vaga*, which had been inferred as relatives by Simidu et al. (19) on the basis of sequence similarity of 16S rRNA. 16S rRNA is not suitable for establishing relationships at this level. The data in Table 2 indicate that the type strain of *A. tetraodonis* is highly related to *A. haloplanktis* strains. We conclude that the name *A. tetraodonis* Simidu et al. 1990 should be rejected because it is a junior subjective synonym of *A. haloplanktis* (ZoBell and Upham 1944) Reichelt and Baumann 1973.

When Chan et al. (9) described *A. undina* as a new species, the closest relative was reported to be *A. haloplanktis* on the basis of numerical analysis of phenotypic features. The homology values presented here (39 to 62%) support their finding from the standpoint of genotypic similarity.

Our results indicate three strains maintained in culture collections as *A. haloplanktis*. Strain IAM 12919 (= NCIMB 19 and ATCC 19855), which was previously identified as a *Pseudomonas* sp., has been extensively used in nutritional and cell structure studies (7) and appears to belong to *A. nigrifaciens*. Strains IAM 12918 (= ATCC 27127) and IAM 13161 (= ATCC 25013) also prove to be misnamed. However, more-extensive taxonomic investigation, including phenotypic characterization, is necessary for conclusive assignments.

Among the species *A. atlantica*, *A. espejiana*, *A. carrageenovora*, *A. haloplanktis*, *A. undina*, and *A. nigrifaciens*, which are placed on the *A. haloplanktis* rRNA branch (22), 18 to 62% homology values were observed. Such values can be considered the indices of the relationships between species in a same genus under the condition used in this study. Homology values between DNAs from *A. macleodii* IAM 12920^T and other *Alteromonas* strains were from 2 to 8%. These values were, under the tested conditions, as low as those between the unrelated strains *E. coli* and *Alteromonas* strains and as those between the strains belonging to different rRNA branches according to the criteria of Van Landschoot and De Ley (22).

Consideration should be given to dividing the genus *Alteromonas* into at least two genera. If that were done, the

generic name *Alteromonas* would have to be restricted to *A. macleodii*.

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