Phylogenetic Analysis of the Genera Alteromonas, Shewanella, and Moritella Using Genes Coding for Small-Subunit rRNA Sequences and Division of the Genus Alteromonas into Two Genera, Alteromonas (Emended) and Pseudoalteromonas gen. nov., and Proposal of Twelve New Species Combinations

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Small-subunit ribosomal DNA sequences were determined for 17 strains belonging to the genera Alteromonas, Shewanella, Vibrio, and Pseudomonas, and these sequences were analyzed by phylogenetic methods. The resulting data confirmed the existence of the genera Shewanella and Moritella, but suggested that the genus Alteromonas should be split into two genera. We propose that a new genus, Pseudoalteromonas, should be created to accommodate 11 species that were previously Alteromonas species, including Pseudoalteromonas atlantica comb. nov., Pseudoalteromonas aurantia comb. nov., Pseudoalteromonas carrageenovora comb. nov., Pseudoalteromonas citrea comb. nov., Pseudoalteromonas denitrificans comb. nov., Pseudoalteromonas espejiana comb. nov., Pseudoalteromonas haloplanktis comb. nov. (with two subspecies, Pseudoalteromonas haloplanktis subsp. haloplanktis comb. nov. and Pseudoalteromonas haloplanktis subsp. tetraodonis comb. nov.), Pseudoalteromonas luteoviolacea comb. nov., Pseudoalteromonas nigricans comb. nov., Pseudoalteromonas rubra comb. nov., and one species that previously was placed in the genus Pseudomonas, Pseudoalteromonas piscicida comb. nov. We propose that P. haloplanktis (type strain, ATCC 14393) should be the type species of the genus Pseudoalteromonas. At this time the emended genus Alteromonas is restricted to a single species, Alteromonas macleodii.

Originally, the genus Alteromonas (4) consisted of four gram-negative, aerobic, nonpigmented, polarly flagellated species of marine bacteria, Alteromonas macleodii (the type species of the genus), Alteromonas vaga, Alteromonas communis, and Alteromonas marinopraesens. The name of the last species was later changed to Alteromonas haloplanktis (31). Subsequently, the genus Alteromonas was often used as a refuge for gram-negative, heterotrophic, aerobic bacteria with single polar flagella which differed from members of the genus Pseudomonas mainly in DNA G+C content (38 to 50 mol%, compared with 55 to 64 mol% for Pseudomonas spp.). As a result, 14 species were assigned to the genus Alteromonas (Table 1). In addition, on the basis of its nonfermentative metabolism, flagellar arrangement, and quinone composition, it was suggested that Pseudomonas piscicida (8) should be included in the genus Alteromonas (1, 6).

rRNA-DNA hybridization experiments (39) revealed that there was a high level of heterogeneity in the genus Alteromonas and that the following three rRNA groups could be distinguished: (i) Alteromonas macleodii, (ii) an Alteromonas haloplanktis cluster containing most Alteromonas species and Pseudomonas piscicida, and (iii) a group containing Alteromonas putrefaciens and Alteromonas hanedai. Alteromonas vaga and Alteromonas communis, which formed a distinct rRNA branch, were transferred to a new genus, the genus Marinomonas. Three species, Alteromonas putrefaciens, Alteromonas hanedai, and Alteromonas colwelliana, were subsequently reassigned to the new genus Shewanella on the basis of the results of a SS rRNA sequence analysis (10, 28), and Alteromonas tetraodonis was reclassified as Alteromonas haloplanktis (2).

Finally, the very low levels of genomic DNA homology between Alteromonas macleodii and all of the other species of the genus confirmed that these organisms were not related (2).

In this study we determined the nearly complete sequences of small-subunit ribosomal genes of 17 strains belonging to the genus Alteromonas and related genera (the genera Shewanella, Vibrio and Pseudomonas) to characterize more precisely the intra- and inter-generic relationships discussed above. These sequences were aligned by comparing them with other eubacterial small-subunit ribosomal DNA (rDNA) sequences, and phylogenetic relationships were determined by using different phylogenetic methods (the maximum-likelihood, maximum-parsimony, and neighbor-joining methods) to check the reliability of each topology. Each topology was then examined by performing a bootstrap analysis to assess its robustness.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains used in this study are listed in Table 2. The bacteria were grown at 22°C on marine agar 2216 (Difco Laboratories, Detroit, Mich.) or were stored frozen at −70°C in marine broth (Difco) supplemented with 20% (vol/vol) glycerol.

DNA amplification and sequencing. The method used to prepare bacterial DNA for PCR was derived from the method of Sritical and Barker (35). Colonies grown on marine agar were suspended in 200 μL of lysis mixture (10 mM Tris [pH 8.0], 1 mM EDTA, 1% Triton X-100) and boiled for 5 min. Following a single cleavage reaction, 5 μL of supernatant was used to amplify small-subunit rDNA as previously described (32). The amplification reaction produced 1.5-kb DNA molecules. After purification on a 1% (wt/vol) agarose gel, the PCR products were directly sequenced as described previously (32). This method yielded large differences in the rDNA sequence corresponding to positions 29 to 1,425 of the Escherichia coli sequence for each representative of the genera Alteromonas and Shewanella, as well as Pseudomonas putrefaciens and Vibrio marina. The sequence of one Alteromonas haloplanktis strain (ATCC 14393) [T = type strain] has been published previously (21).

Phylogenetic analysis. Sequences were aligned and studied by using a set of programs developed in our laboratory (available from R. Christen). In this...
analysis we used the sequences determined in this study and small-subunit rDNA sequences of the following bacteria which were obtained from the EMBL: Alteromonas haloplanktis, Alteromonas nigrifaciens, A. haloplanktis, Arsenophonus nasoniae, E. coli, Haemophilus ducreyi, Hafnia alvei, Marinobacter hydrocarbonoclasticus, Marinomonas vaga, Pasteurella multocida, Photobacterium angusturn, Vibrio nereis, Alteromonas. We obtained the sequence of a bacterium that has not been isolated. The sequence within the gamma 3 subgroup are shown in Fig. 1, an unrooted tree in which the results of a neighbor-joining analysis (topology shown in Fig. 1) are combined with the results obtained by maximum-likelihood and maximum-parsimony methods. We included representatives of all of the major taxa previously identified as members of the gamma 3 clade (the families Vibrionaceae, Enterobacteriaceae, Pasteurellaceae, and Aeromonadaceae) in these analyses.

**RESULTS AND DISCUSSION**

All of the sequences studied were aligned by comparing them with a database containing about 3,000 aligned eubacterial small-subunit rDNA sequences. The results of a broad phylogenetic analysis clearly confirmed that all of the species which we studied belonged to the gamma subgroup of the phylum Proteobacteria of the Eubacteria (data not shown) and, more precisely, to the well-defined robust monophyletic taxon also designated the gamma 3 subgroup (15, 32, 42). The phylogenetic positions of the genera *Alteromonas* and *Shewanella* within the gamma 3 subgroup are shown in Fig. 1, an unrooted tree in which the results of a neighbor-joining analysis (topology shown in Fig. 1) are combined with the results obtained by maximum-likelihood and maximum-parsimony methods. We included representatives of all of the major taxa previously identified as members of the gamma 3 clade (the families Vibrionaceae, Enterobacteriaceae, Pasteurellaceae, and Aeromonadaceae) in these analyses.

The results of all of the analyses confirmed that the three *Shewanella* species form an independent clade that can be recognized as a genus (Fig. 1). All of these species formed a robust monophyletic taxon (as determined by all methods and 81% of the bootstrap replications) that branched deeply and did not cluster with any other sequence. *Shewanella hanedai* and *Shewanella benthica* were closely related as determined by all three methods, a result supported by 92% of the bootstrap replications. Therefore, our data confirmed the results of previous rRNA-DNA hybridization experiments (39) and SS rRNA (28) and partial small-subunit rRNA sequence (24) analyses.

Figure 1 shows that the sequence of *V. marinus* did not cluster with the sequence of any other member of the *Vibrio*aceae available at this time (32). The separate position of *V. marinus* has also been observed in SS rRNA sequence studies.
(27, 28), partial small-subunit rRNA sequence studies (24), and DNA-DNA relatedness studies (36). Therefore, it has been proposed that *V. marinus* should be renamed *Mortiella marinus* (36). Our complete *V. marinus* sequence matched a previously published partial sequence (24) but not the sequence determined by Ruimy et al. (32). As discussed by Ruimy et al., their sequence is probably not the sequence of *V. marinus*. On the basis of its true sequence, *V. marinus* branched deeply and did not cluster with any other genus, despite a slight association with the genus *Shewanella* and the “*Alteromonas*” cluster. The deep branching and the lack of association with any other genus confirmed that this bacterium should be placed in a separate genus. Therefore, we support the proposal to create the genus *Mortiella*, which contains the single species *Mortiella marinus* (36).

*Alteromonas macleodii* clearly did not belong to the monophyletic taxon which included all of the other “*Alteromonas*” species (Fig. 1). *Alteromonas macleodii* branched deeply and did not cluster with any other organism whose sequence was available. All of the other “*Alteromonas*” species, *Pseudoalteromonas piscicida*, and the unnamed bacterium whose nucleotide sequence accession number was Z25522 and *Pseudoalteromonas denitrificans* appeared to resemble the majority of the species. The last group included closely related species, and the phylogenetic relationships of these species were difficult to determine on the basis of small-subunit rRNA cistron similarity data (39) and the isoprenoid quinone compositions of the organisms (1). Finally, because of its nonfermentative metabolism and flagellar arrangement, *Pseudoalteromonas piscicida* appeared to resemble the majority of the species of this genus phenotypically (6). Because this bacterium undoubtedly belongs to the new genus *Pseudoalteromonas*, the generic name *Pseudoalteromonas* should not be used for it any longer. Thus, it is appropriate to rename this bacterium *Pseudoalteromonas piscicida* gen. nov., comb. nov.

Within the new genus *Pseudoalteromonas*, phylogenetic relationships were difficult to resolve when distant outgroups were included, as in Fig. 1. Nevertheless, we distinguished (Fig. 1) two deeply branched species (the bacterium whose nucleotide sequence accession number was ATCC27126 and *Pseudoalteromonas denitrificans*) that were clearly outgroups with respect to all of the other species (as determined by all three methods and 75% of the bootstrap replications). A more detailed phylogenetic analysis of the *Pseudoalteromonas* cluster was performed by using *Pseudoalteromonas denitrificans* as the outgroup (Fig. 2). The new genus *Pseudoalteromonas* could be divided into the following four monophyletic taxa, which were identified by all three phylogenetic methods: (i) *Pseudoalteromonas denitrificans*; (ii) two pigmented species, *Pseudoalteromonas citrea* and *Pseudoalteromonas auranta* (100% of the bootstrap values); (iii) three other pigmented species, *Pseudoalteromonas piscicida*, *Pseudoalteromonas rubra*, and *Pseudoalteromonas luteoviolacea* (98% of the bootstrap values); and (iv) all nonpigmented *Pseudoalteromonas* species (84% of the bootstrap values). The last group included closely related species, and the phylogenetic relationships of these taxa were difficult to determine on the basis of small-subunit rDNA sequences. Within the nonpigmented *Pseudoalteromon-
FIG. 1. Phylogenetic positions of Alteromonas, Moritella, and Shewanella species within the gamma 3 subgroup of the phylum Proteobacteria. An unrooted phylogenetic tree was obtained by performing a neighbor-joining analysis; branches that were significantly positive at a level of $P < 0.01$ as determined by a maximum-likelihood method are indicated by two asterisks. There are numbers above the branches that were also identified by the maximum-parsimony method (most parsimonious tree), and these numbers indicate how the branches were supported by the bootstrap analysis results. The sequences of the underlined species were determined in this study. Marizobacter hydrocarbonoclasticus and Marinomonas vaga were used as outgroups for the gamma 3 subgroup of the Proteobacteria.

nas species group, DNA-DNA hybridization experiments revealed that Pseudoalteromonas haloplanktis ATCC 14393 and Pseudoalteromonas haloplanktis subsp. tetrodonis ATCC 51193 exhibited levels of relatedness ranging from 82 to 84% (2). Considering that there were a number of differences between the small-subunit rDNA sequences of these organisms (14 differences in 1,429 nucleotides), that biochemical analyses revealed a number of traits which can be used to differentiate these taxa (Table 3), and that their level of genomic DNA relatedness is less than 85%, we propose that they should be placed in different subspecies. Thus, we propose that strain ATCC 51193 is a Pseudoalteromonas haloplanktis subsp. tetrodonis comb. nov. strain and that strain ATCC 14393 is a Pseudoalteromonas haloplanktis subsp. haloplanktis comb. nov. strain.

We propose that Pseudoalteromonas haloplanktis (type strain, ATCC 14393) should be the type species of the genus Pseudoalteromonas because (i) it was the first species described in this new genus (31), (ii) it has been used more widely than any other species for laboratory studies of marine bacteria (13,
29, 38), (iii) it is nonpigmented (most likely an ancestral characteristic of this genus), and (iv) it is centrally located in molecular phylogenies.

Finally, the bacterium whose sequence has been deposited under accession number Z25522 in the EMBL data bank, which has not been isolated in culture yet, clustered with the new genus *Pseudoalteromonas*, but a name cannot be proposed since the phenotype of this organism is not known.

**Description of the genus *Pseudoalteromonas*** gen. nov. *Pseudoalteromonas* (Pseu. do. al. te. ro. mon’ as. Gr. adj. pseudes, false; L.n. Alteromonas, genus of gram-negative, aerobic, marine bacteria; L.n. Pseudoalteromonas, false Alteromonas). The phenotypic description of the genus *Pseudoalteromonas* is the same as the description published previously in *Bergey’s Manual of Systematic Bacteriology* (6) and *The Prokaryotes* (20) for the genus *Alteromonas*, except for traits that are specific to *Alteromonas macleodii* (see below). The cells of all *Pseudoalteromonas* species are gram-negative, non-spore-forming, straight or curved rods that are 0.2 to 1.5 by 1.8 to 3 μm. The cells of most species are motile by means of single unsheathed polar flagella; *Pseudoalteromonas luteoviolacea* and *Pseudoalteromonas denitrificans* have sheathed flagella. Not luminescent. Several species produce pigments. Strictly aerobic. Chemoorganotrophs with respiratory but not fermentative metabolism. Oxidase positive. Catalase activity is generally weak and irregular. All species grow at 20°C. Only one species (*Pseudoalteromonas denitrificans*) is capable of denitrification. None of the strains has a constitutive arginine dihydrolase system. Strains do not accumulate poly-p-hydroxybutyrate. All species require a seawater base for growth. Many strains require organic growth factors. The following combination of properties is found in all 12 known species: positive for gelatinase, lipase, lecithinase, and DNase activities and utilization of D-glucose as a sole source of carbon; and negative for utilization of D-ribose, L-rhamnose, turanose, salicin, D-glucuronate, DL-glycerate, erythritol, sorbitol, meso-inositol, adonitol, L-valine, L-ornithine, and m-hydroxybenzoate. The G+C content of the DNA ranges from 37 to 50 mol%.

The type species is *Pseudoalteromonas haloplanktis*; the type strain of this species is strain ATCC 14393 (= strain 215 of Baumann et al. [4]).


**Description of *Pseudoalteromonas haloplanktis* subsp. *haloplanktis* (ZoBell and Upham) comb. nov.** The description of *Pseudoalteromonas haloplanktis* subsp. *haloplanktis* comb. nov.
is identical to the description given by ZoBell and Upham (45). The type strain is strain ATCC 14392.

Description of Pseudoalteromonas haloplankts subsp. tetraodonis (Simidu, Kita-Tsuchamoto, Yasumoto, and Yotsu) comb. nov. The description of Pseudoalteromonas haloplankts subsp. tetraodonis comb. nov. is identical to the description given by Simidu et al. (34). The type strain is strain ATCC 51193.

Description of Pseudoalteromonas atlantica (Akagawa-Matsushita, Matsu, Koga, and Yamasato) comb. nov. The description of Pseudoalteromonas atlantica comb. nov. is identical to the description given by Akagawa-Matsushita et al. (3). The type strain is strain ATCC 19262.

Description of Pseudoalteromonas aurantia (Gauthier and Breittmayer) comb. nov. The description of Pseudoalteromonas aurantia comb. nov. is identical to the description given by Gauthier and Breittmayer (19). The type strain is strain ATCC 33046.

Description of Pseudoalteromonas carrageenovora (Akagawa-Matsushita, Matsu, Koga, and Yamasato) comb. nov. The description of Pseudoalteromonas carrageenovora comb. nov. is identical to the description given by Akagawa-Matsushita et al. (3). The type strain is strain ATCC 43555.

Description of Pseudoalteromonas citrea (Gauthier) comb. nov. The description of Pseudoalteromonas citrea comb. nov. is identical to the description given by Gauthier (17). The type strain is strain ATCC 29719.

Description of Pseudoalteromonas denitrificans (Enger, Nygaard, Solberg, Schei, Nielsen, and Dandus) comb. nov. The description of Pseudoalteromonas denitrificans comb. nov. is identical to the description given by Enger et al. (12). The type strain is strain ATCC 43337.

Description of Pseudoalteromonas espejiana (Chan, Baumann, Garza, and Baumann) comb. nov. The description of Pseudoalteromonas espejiana comb. nov. is identical to the description given by Chan et al. (9). The type strain is strain ATCC 29659.

Description of Pseudoalteromonas luteoviolacea (Gauthier) comb. nov. The description of Pseudoalteromonas luteoviolacea comb. nov. is identical to the description given by Gauthier (18). The type strain is strain ATCC 33492.

Description of Pseudoalteromonas nigrofaciens (White) comb. nov. The description of Pseudoalteromonas nigrofaciens comb. nov. is identical to the description given by White (41). The type strain is strain ATCC 19375.

Description of Pseudoalteromonas rubra (Gauthier) comb. nov. The description of Pseudoalteromonas rubra comb. nov. is identical to the description given by Gauthier (16). The type strain is strain ATCC 29570.

Description of Pseudoalteromonas undina (Chan, Baumann, Garza, and Baumann) comb. nov. The description of Pseudoalteromonas undina comb. nov. is identical to the description given by Chan et al. (9). The type strain is strain ATCC 29660.

Emended description of the genus Alteromonas, Gram-negative, non-spore-forming straight rods that are 0.7 to 1 μm in diameter and 2 to 3 μm long. Motile by means of a single unsheathed polar flagellum. Not luminescent and not pigmented. Strictly aerobic. Chemoheterotroph with respiratory but not fermentative metabolism. Oxidase positive and catalase negative. Growth occurs at 20 to 35°C but not at 4°C. Does not denitrify. No constitutive arginine dilydrolyase system. Does not accumulate poly-β-hydroxybutyrate from the monomer β-hydroxybutyrate. Requires a seawater base for growth, but not organic growth factors. The G+C content of the DNA is 44 to 47 mol%.

The type species is Alteromonas macleodii, whose type strain is strain ATCC 27126 (= strain 107 of Baumann et al. [4]).

The characteristics of the type species are the same as those of the genus.

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REFERENCES