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Phylogenetic relationships among marine *Alteromonas*-like proteobacteria: emended description of the family *Alteromonadaceae* and proposal of *Pseudoalteromonadaceae* fam. nov., *Colwelliaceae* fam. nov., *Shewanellaceae* fam. nov., *Moritellaceae* fam. nov., *Ferrimonadaceae* fam. nov., *Idiomarinaceae* fam. nov. and *Psychromonadaceae* fam. nov.

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The phylogenetic relationships among marine *Alteromonas*-like bacteria of the genera *Alteromonas*, *Pseudoalteromona*s, *Glaciecola*, *Thalassomonas*, *Colwellia*, *Idiomarina*, *Oceanimonas*, *Oceanisphaera*, *Shewanella*, *Moritella*, *Ferrimonas*, *Psychromonas* and several other genera of the '*Gammaproteobacteria*' were studied. Results of 16S rRNA gene sequence analyses revealed that some members of these genera formed several coherent groups at the family level. Characteristic signature oligonucleotides for studied taxa were defined. Signature positions are divided into three classes: (i) single compensatory mutations, (ii) double compensatory mutations and (iii) mutations affecting nucleotides not paired in the secondary structure. The 16S rRNA gene sequence similarity level within genera was 93 % or above. This value can be a useful additional criterion for genus discrimination. On the basis of this work and previous polyphasic taxonomic studies, the circumscription of the family *Alteromonadaceae* is limited to the genera *Alteromonas* and *Glaciecola* and the creation is proposed of the families *Pseudoalteromonadaceae* fam. nov. to accommodate bacteria of the genera *Pseudoalteromonas* and *Algicola* gen. nov. (formerly *Pseudoalteromonas bacteriolytica*) and *Colwelliaceae* fam. nov. to accommodate bacteria of the genera *Colwellia* and *Thalassomonas*. Bacteria of the genera *Oceanimonas* and *Oceanisphaera* formed a robust cluster and shared common signature oligonucleotides. Because of deep branching and lack of association with any other genus, the following families are proposed that include single genera: *Idiomarinaceae* fam. nov., *Psychromonadaceae* fam. nov., *Moritellaceae* fam. nov., *Ferrimonadaceae* fam. nov. and *Shewanellaceae* fam. nov. Finally, this study also revealed that [*Hyphomicrobium*] *indicum* should be reclassified as *Photobacterium indicum* comb. nov.

INTRODUCTION

A large group of Gram-negative marine heterotrophic bacteria of the genera *Alteromonas*, *Pseudoalteromonas*, *Glaciecola*, *Thalassomonas*, *Colwellia*, *Idiomarina*, *Shewanella*, *Moritella*, *Ferrimonas* and *Psychromonas* that are tentatively considered as *Alteromonas*-like or *Alteromonas*-related bacteria belong to the class '*Gammaproteobacteria*' of the recently proposed phylum *Proteobacteria* (Garrity & Holt,

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; OGU, operational genetic unit; OTU, operational taxonomic unit; PUFA, polyunsaturated fatty acid.

A 16S rRNA gene sequence similarity matrix and an analysis of the distribution of similarities, a global tree resulting from large-scale neighbour-joining analysis and detailed trees of species of *Pseudoalteromonas*, *Alteromonas* and *Colwellia* are available as supplementary material in IJSEM Online.

2001; Garrity *et al.*, 2002; Cavalier-Smith, 2002) that was derived from the elevated class *Proteobacteria* Stackebrandt *et al.* 1988. Many of these bacteria have similar morphological, physiological and biochemical features, which impedes their identification. The taxonomic history of *Alteromonas*-related bacteria is a reflection of dynamic changes in bacterial systematics due to the rapid development of phylogenetic analysis and molecular techniques. The first volume of *Bergey's Manual of Systematic Bacteriology* (Baumann *et al.*, 1984b) described only one genus of Gram-negative, aerobic, heterotrophic, marine bacteria with one polar flagellum, *Alteromonas* Baumann *et al.* 1972, whose members were phenotypically similar to pseudomonads, but differed from them in the lower G+C content of their DNA. This genus originally included few species: *Alteromonas macleodii*, *A. haloplanktis*, '*A. marinopraesens*' (reclassified as *A. haloplanktis*; Reichelt & Baumann, 1973), [*A.*] *communis* and [*A.*] *vaga* (Gauthier & Breittmayer, 1992). Later, a number of other species were described, namely *Alteromonas rubra*, *A. citrea*, *A. luteoviolacea*, *A. aurantia* (Gauthier & Breittmayer, 1992), *A. espejiana*, *A. undina* (Chan *et al.*, 1978), [*A.*] *putrefaciens* (Lee *et al.*, 1981), '*A. thalassomethanolica*' (Yamamoto *et al.*, 1980) and *A. nigrifaciens* (White, 1940; Baumann *et al.*, 1984a; Ivanova *et al.*, 1996a). Intensive rRNA–DNA hybridization study (Van Landschoot & De Ley, 1983) showed a high level of genetic heterogeneity among the members of the genus and allowed the following rRNA relatedness clusters to be revealed: (i) the *A. macleodii* cluster; (ii) the *A. haloplanktis* cluster, which included the majority of *Alteromonas* species and one species from the genus *Pseudomonas*, [*Pseudomonas*] *piscicida* (Bein, 1954; Buck *et al.*, 1963); (iii) the [*A.*] *putrefaciens* and [*Alteromonas*] *hanedai* cluster (Jenson *et al.*, 1983); and (iv) the [*A.*] *vaga* and [*A.*] *communis* cluster, which was classified as a new genus, *Marinomonas* (Gauthier & Breittmayer, 1992). Based on 5S rRNA sequences, the species [*A.*] *putrefaciens*, [*A.*] *hanedai* and [*Alteromonas*] *colwelliana* (Weiner *et al.*, 1988) were combined into a new genus, *Shewanella* (MacDonell & Colwell, 1985). By the early 1990s, the genus *Alteromonas* had been supplemented with several novel species, *Alteromonas denitrificans* (Enger *et al.*, 1987), *A. atlantica*, *A. carrageenovora* (Akagawa-Matsushita *et al.*, 1992), *A. tetraodonis* (Simidu *et al.*, 1990), '*A. rava*' (Kodama *et al.*, 1993), [*A.*] *fuliginea*, *A. distincta* and *A. elyakovii* (Romanenko *et al.*, 1994, 1995; Ivanova *et al.*, 1996b).

In 1995, based on the analysis of 16S rRNA gene sequences, the genus *Alteromonas* was revised. The revised genus *Alteromonas* contained only one species, *A. macleodii*, while a new genus *Pseudoalteromonas*, which included the rRNA relatedness group II species, was formed (Gauthier *et al.*, 1995). According to DNA–DNA hybridization data and phylogenetic studies, the name *A. fuliginea* should be regarded as a later synonym of the name *Pseudoalteromonas citrea* (Ivanova *et al.*, 1998), while the species *A. distincta* and *A. elyakovii* were transferred to the genus *Pseudoalteromonas* (Ivanova *et al.*, 2000a; Sawabe *et al.*, 2000). The species *A. tetraodonis* has been reclassified as *A. haloplanktis* subsp. *tetraodonis* (Akagawa-Matsushita *et al.*, 1993). However, further studies showed that this species had to be retrieved, as *Pseudoalteromonas tetraodonis* (Ivanova *et al.*, 2001a). In recent years, a number of novel species of marine pseudoalteromonads have been described, such as *Pseudoalteromonas antarctica* (Bozal *et al.*, 1997) and *Pseudoalteromonas prydzensis* (Bowman, 1998), which was isolated from Antarctic coastal waters, *Pseudoalteromonas bacteriolytica* (Sawabe *et al.*, 1998), which was isolated from wounded fronds of *Laminaria japonica* collected from the Sea of Japan, and *Pseudoalteromonas peptidolytica* (Venkateswaran & Dohmoto, 2000), which was isolated from sea water. The highly bioactive species *Pseudoalteromonas tunicata* (Holmström *et al.*, 1998) was isolated from the ascidian *Ciona intestinalis* residing in coastal waters of western Sweden. More recently, several more species were proposed, including *Pseudoalteromonas ulvae* (Egan *et al.*, 2001), *Pseudoalteromonas issachenkonii* (Ivanova *et al.*, 2002a), *Pseudoalteromonas ruthenica* (Ivanova *et al.*, 2002b), *Pseudoalteromonas maricaloris*, *Pseudoalteromonas flavipulchra* (former *A. aurantica* NCIMB 2033) (Ivanova *et al.*, 2002c), *Pseudoalteromonas translucida*, *Pseudoalteromonas paragorgicola* (Ivanova *et al.*, 2002d), *Pseudoalteromonas agarivorans* (Romanenko *et al.*, 2003a) and *Pseudoalteromonas phenolytica* (Isnansetyo & Kamei, 2003).

Bowman *et al.* (1998b) described a group of pigmented, psychrophilic, strictly aerobic, heterotrophic organisms isolated from sea-ice cores from eastern Antarctica that formed a distinct branch adjacent to *Alteromonas*. These bacteria received genus status and consisted of two species, *Glaciecola punicea* and *Glaciecola pallidula*. One more species, *Glaciecola mesophila*, recently enlarged the genus (Romanenko *et al.*, 2003b).

A few years later, the aerobic marine genus *Idiomarina* was described, which included two species, *Idiomarina abyssalis* and *I. zobellii* (Ivanova *et al.*, 2000a). These bacteria were isolated from sea-water samples taken from depths of 4000 and 5000 m, respectively. The species were phenotypically close to bacteria of the genera *Alteromonas*, *Pseudoalteromonas* and *Marinomonas*, but differed from them in their cellular fatty acid profiles and their inability to use carbohydrates as sole sources of carbon and energy. The two species were distinguished by their characteristic morphology: *I. zobellii* cells were fimbriated, while *I. abyssalis* cells were enclosed in sheaths. Recently two more species were described, *Idiomarina baltica* (Brettar *et al.*, 2003) and *I. loihiensis* (Donachie *et al.*, 2003).

The genus *Colwellia* (Deming *et al.*, 1988) originally included two facultatively anaerobic bacteria, *Colwellia psychrerythraea* and *C. hadaliensis*. The first strains of this genus were isolated from water samples taken in the Mariana Trench and near the coast of the United States. The type strain of the species *C. psychrerythraea* was found to be an obligate barophile. Bowman *et al.* (1998a) described four novel psychrophilic species of this genus, *Colwellia*

demingiae, *C. hornerae*, *C. rossensis* and *C. psychrotropica*, and novel strains of *C. psychrerythraea*. None of these Antarctic isolates were barophilic and all of them synthesized docosahexaenoic acid (22 : 6ω3) in amounts of up to 8 % of the total cellular content of fatty acids. The type strain of the species *Colwellia maris* was originally assigned to the genus *Vibrio* and was subsequently reclassified (Yumoto *et al.*, 1998).

Another genus, closely related to genus *Colwellia*, *Thalassomonas*, represented by a single species *Thalassomonas viridans*, was described to accommodate halophilic chemoorganotrophic bacteria isolated from oysters cultivated off the Mediterranean coast at Valencia (Spain) (Macián *et al.*, 2001).

Currently, the genus *Shewanella* MacDonell and Colwell 1985 comprises more than 20 species (*Shewanella algae*, *S. amazonensis*, *S. baltica*, *S. benthica*, *S. colwelliana*, *S. denitrificans*, *S. fidelis*, *S. frigidimarina*, *S. gelidimarina*, *S. hanedai*, *S. japonica*, *S. livingstonensis*, *S. marinintestina*, '*S. massilia*', *S. olleyana*, *S. oneidensis*, *S. pealeana*, *S. putrefaciens*, *S. sairae*, *S. schlegeliana*, *S. violacea*, *S. waksmanii* and *S. woodyi*). These species are Gram-negative, facultatively anaerobic and aerobic, readily cultivated gammaproteobacteria mainly associated with aquatic habitats (Jensen *et al.*, 1980; Lee *et al.*, 1981; Weiner *et al.*, 1988; Coyne *et al.*, 1989; Gauthier *et al.*, 1995; Bowman *et al.*, 1997; Leonardo *et al.*, 1999; Venkateswaran *et al.*, 1999; Ivanova *et al.*, 2001b, 2003; Bozal *et al.*, 2002; Satomi *et al.*, 2003). During the last decade, bacteria of this genus have been studied extensively because of their important role in co-metabolic bioremediation of halogenated organic pollutants, destructive souring of crude petroleum and the dissimilatory reduction of magnesium and iron oxides and their ability to produce high proportions of polyunsaturated fatty acids (PUFA) (Myers & Nealson, 1988; Petrovskis *et al.*, 1994; Russell & Nichols, 1999).

The closest relatives of *Shewanella* species are bacteria of the genus *Moritella* Urakawa *et al.* 1998 that are represented by four species, *Moritella marina* (Urakawa *et al.*, 1998), *M. japonica* (Nogi *et al.*, 1998), *M. yayanosii* (Nogi & Kato, 1999) and *M. viscosa* (Benediktsdóttir *et al.*, 2000).

Because of the initial phenotypic misclassification of [*Pseudomonas*] *doudoroffii* as a close relative of *Aeromonas hydrophila* and *Tolumonas auensis*, its taxonomic status and phylogenetic relationships within the '*Gammaproteobacteria*' remained unclear until recently. Brown *et al.* (2001) proposed to create the genus *Oceanimonas* to accommodate the novel phenol-degrading bacterium *Oceanimonas baumannii* as well as [*Pseudomonas*] *doudoroffii* (Brown *et al.*, 2001).

The final two genera comprise marine facultatively anaerobic and aerotolerant anaerobic gammaproteobacteria: *Ferrimonas*, represented so far by the species *Ferrimonas balearica* (Rosselló-Mora *et al.*, 1995), and *Psychromonas*, which possesses five species *Psychromonas antarctica* (Mountfort *et al.*, 1998), *Psychromonas kaikoae* (Nogi *et al.*, 2002), *Psychromonas marina* (Kawasaki *et al.*, 2002), *Psychromonas arctica* (Groudieva *et al.*, 2003) and *Psychromonas profunda* (Xu *et al.*, 2003).

This study is a further extension of our investigation of *Alteromonas*-like gammaproteobacteria (Ivanova & Mikhailov, 2001) and aimed to provide a basis of a delineation system based on a comprehensive overview of their phylogenetic relationships (16S rRNA gene sequences), the presence of specific compensatory mutations visible in the secondary structure of the molecule and polyphasic classification strategy. This paper mainly reviews published data with reference to the relevant publications that contain original or cited data on phenotypic and chemotaxonomic characteristics used in the identification of *Alteromonas*-like bacteria.

METHODS

16S rRNA gene sequence selection. The core of our analysis were the available 16S rRNA gene sequences of *Alteromonas*-like bacteria and selected relatives of the '*Gammaproteobacteria*'. Sequences were selected within a local database of 81 156 already-aligned sequences (corresponding roughly to release 74, May 2003), of which 56 267 had a length of more than 500 nt and 33 943 a length of more than 1000 nt. In order to derive proper phylogenetic analyses, only these longer sequences were considered. These sequences included 13 780 members of the *Proteobacteria*, among which 6094 were members of the '*Gammaproteobacteria*'. Among these last sequences, about 2550 were identified at the species level (i.e. were not described as unidentified species, clones, bacteria sp., etc.). Large-scale phylogenetic analyses (neighbour-joining; NJ) using these last sequences allowed the identification of genera that had close relationships to the marine bacteria analysed (see the global tree available as Supplementary Fig. A in IJSEM Online). In the final analysis of this work (see below), we retained 24 species of *Pseudoalteromonas* (see Supplementary Fig. B), three species of *Alteromonas* (see Supplementary Fig. C), two species of *Glaciecola*, four species of *Idiomarina*, 18 species of *Shewanella*, five species of *Moritella*, seven species of *Colwellia* (see Supplementary Fig. D), four species of *Psychromonas*, *Ferrimonas balearica* and some neighbouring species; in total, 119 sequences. Selection was according to their placement in the global tree and availability of sequences for type strains. Strain numbers and their corresponding accession numbers are indicated on the phylogenetic trees.

Phylogenetic analyses. Phylogenetic dendrograms were reconstructed according to three different methods: NJ (BIONJ), maximum-likelihood (ML) (using the Global option) and maximum-parsimony (MP). For the NJ analysis, a matrix distance was calculated according to Kimura's two-parameter correction. Bootstraps were done using 1000 replications, BIONJ and Kimura's two-parameter correction. BIONJ was according to Gascuel (1997), ML and MP were from PHYLIP (Phylogeny Inference Package, version 3.573c; distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle, USA). Preliminary phylogenetic analyses were done using the most conserved parts of the sequences. Phylogenetic dendrograms were drawn using NJPLOT (Perrière & Gouy, 1996).

Domains used to construct final phylogenetic trees excluded positions likely to show homoplasy or that were difficult to align. When bacterial

sequences from different genera are used to determine phylogenetic relationships, domains used to construct a phylogenetic tree should be examined extremely carefully, since positions that can be properly aligned decrease and homoplasy increases with the depth of the phylogenetic tree. For that reason, a detailed phylogenetic tree that analyses the position of a species within a genus is usually different from that used to position this genus within its class or phylum. As a result, the tree presented in this paper (and the trees available as supplementary material) should be taken with caution near their leaves: the analysis has been done to position the genera in the '*Gammaproteobacteria*', not to position the different species within a genus. These trees define which species belong to a genus, but not, with consistency, which are sister species. For Fig. 1, the domains used corresponded to positions 95–175, 193–442, 454–819 and 845–1393 of the sequence of *Aeromonas allosaccharophila* CECT 4199^T (S39232). The topology shown is that of the bootstrap analysis, as it as been demonstrated that this topology is often better than that of a simple tree (Berry & Gascuel, 1996).

Operational genetic unit (OGU) analysis. We have also analysed how phylogenetic clusters could be interpreted in terms of groups of sequences sharing a fixed percentage of similarity. Similarly to OTU (operational taxonomic unit), we defined an OGU as a group of sequences in which every sequence has a similarity above a cut-off level with at least one sequence of this group and similarities under this level with every sequence not included in the group (simple linkage method). Percentages of 16S rRNA gene sequence similarities between each pair of sequences were calculated by parsing the result of a stand-alone BLAST analysis of these sequences on themselves, with the options no filter and W = 7. All non-overlapping high-scoring-segment pairs (see BLAST documentation at NCBI) were taken into account to calculate the percentages of similarity (Supplementary Table A in IJSEM Online). Table 1 shows the numbers of OGUs obtained when the cut-off was increased, and OGUs obtained at 93 % (at which a clear plateau was obtained corresponding largely to genus delimitation) of similarity are indicated in Supplementary Fig. E. In order to perform this comparison, it is necessary to compare only sequences that overlap completely (no missing 5′ or 3′ end); for this reason, similarity values were calculated using positions 137–1335 corresponding to *Aeromonas allosaccharophila* CECT 4199^T (S39232).

Signature nucleotides. For each cluster revealed by the phylogenetic analysis, we have searched for single nucleotides that may be present in every sequence of a specific cluster and absent in any other sequence. This was done using an alignment of the sequences (manually checked) of the *Alteromonas*-like bacteria with that of *Escherichia coli*. A specific program (SeqAm; S. Flavier and R. Christen, unpublished) has been written for that purpose. Every signature nucleotide found was then positioned on the secondary structure of the 16S rRNA molecule of *E. coli* (obtained from http://www.rna.icmb.utexas.edu/members/). This analysis allowed the interpretation of the signatures found in terms of single or double compensatory mutations in helices of the secondary structure. Compensatory mutations are two nucleotides that stabilize a stem in the secondary structure (such as G–C) and that are mutated (for example to C–G) in specific taxa. It must be stated that, if transitions such as G–C to A–T (through the stable intermediate G–T) are common, transversion mutations such as G–C to C–G are comparatively rare and are thus particularly strong signatures. The types of mutations (not involved in stems, simple mutations, double mutations) are indicated in Table 1.

RESULTS AND DISCUSSION

Phylogenetic analyses

Any phylogenetic method has its 'Felsenstein' zone, i.e. can retrieve the wrong tree (even if the bootstrap percentages are high) when the dataset does not fulfil implicit hypotheses on which the method is based. As a result, it is considered that a proper phylogenetic analysis should include the comparison of the results of different methods by combining three algorithms (NJ, MP and ML). Branches in Fig. 1 are labelled to indicate which branches were found by all analyses. Labelled branches are thus particularly 'secure', which does not mean that unlabelled branches are wrong, simply that we cannot be sure that they represent the evolutionary history of the gene.

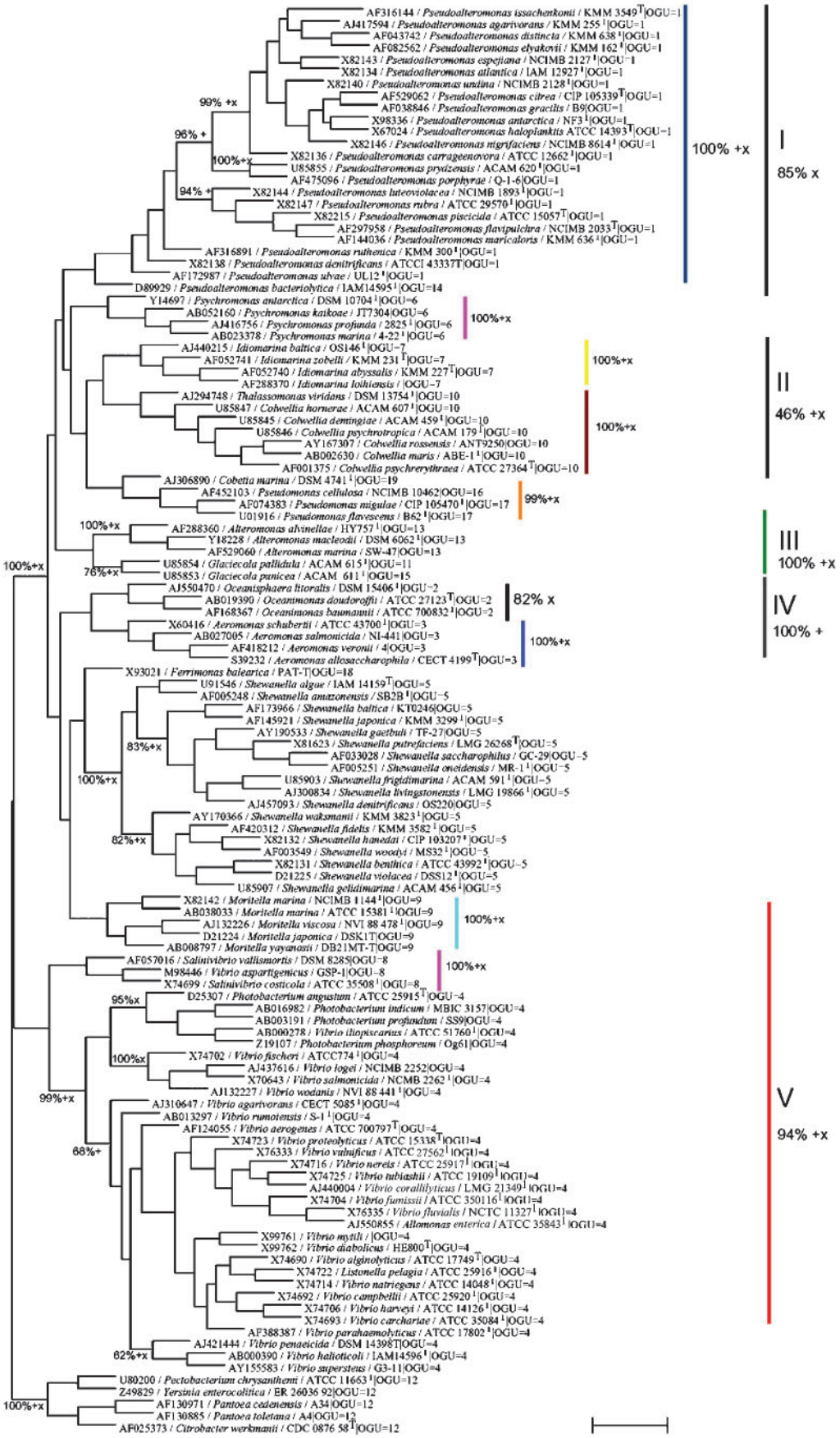
Phylogenetic analyses based on 16S rRNA gene sequences

As discussed below, it would have been best to be able to compare the results of analyses using different genes. However, this was not possible as only 16S rRNA gene sequences are available for representatives of the group of bacteria analysed (see below). Phylogenetic analyses of 16S rRNA gene sequences of the *Alteromonas*-like bacteria revealed that, despite their close phenetic similarity, bacteria of the genera *Alteromonas*, *Pseudoalteromonas*, *Glaciecola*, *Thalassomonas*, *Colwellia* and *Idiomarina* did not form a clade but several phylogenetically distinct lineages that were consistently recovered from several phylogenetic analyses. The topology shown in Fig. 1 is that of the bootstrap tree from the NJ method. Only branches with percentages indicated should be interpreted as consistent. Some other branches were retrieved by two methods only when all sequences were used (for example *Pseudoalteromonas*; see Supplementary Fig. B). This is sometimes a problem with character-based methods (ML or MP) when the number of sequences analysed approaches the number of significant characters; reducing the number of sequences analysed allowed us to find a monophyletic taxon with all three methods (Supplementary Figures B–D in IJSEM Online). Finally, phylogenetic analyses based on a single gene reveal the history of the gene, not always that of the species. 16S rRNA gene sequences are, however, peculiar as they pertain to a true multigene family; their presence usually in multiple copies and the phenomenon of gene conversion (Cilia *et al.*, 1996) render them less sensitive to lateral transfer (no positive selective pressure and unlikely genetic drift) and they are thus particularly appropriate for phylogenetic analyses above the species level (Cilia *et al.*, 1996). It is clear, however, that the precise position of each genus within the phylum *Proteobacteria* will become clearer as more and more housekeeping gene sequences become available, a goal that has not yet been reached for *Alteromonas*-like bacteria.

Table 1. Nature of signature sites detected among the various taxa

Signature sites are shown in bold, other columns indicate the nucleotide present in each OGU. OGUs are numbered as in Fig. 1: 1, *Pseudoalteromonas*; 2, *Oceanimonas*; 3, *Aeromonas*; 4, *Vibrio*; 6, *Psychromonas*; 7, *Idiomarina*; 8, *Salinivibrio* + '*Vibrio aspartigenicus*'; 9, *Moritella* and *Idiomarina*; 10, *Colwellia*; 10b, *Thalassomonas* + *Colwellia hornerae*; 11, 13 and 15, *Alteromonas*/*Glaciecola*; 12, outgroup; 16, 17 and 19, *Pseudomonas*/*Halomonas*. No indication is given for clusters not detailed in the manuscript. Types: 1, double compensatory mutations; 2, single compensatory mutation; 3, non-paired nucleotide. Dashes indicate deletions in some sequences at this position.

OGU	Type	Position	1	2	3	4	6	7	8	9	10	10b	12	11, 13, 15	16, 17, 19
1	1	661	**A**	G	G	G	G	G	G	**T**	G	G−	G	G	G
1	3	733	**A**	G	G	G	G	G	G	G	G	G	G	G	G
1	1	744	**T**	C	C	C	C	C	C	**A**	C	C	C	C	C
1	1	833	**C**	T	G	G	T	T	G	T	T	T	G	T	T
1	2	852	**T**	G	G	G	G	A	G	G	A	G	G	AC	A
1	1	853	**T**	G	C	C	G	G	C	G	G(−)	G	C	G	G
3	1	593	T	T	**A**	T	T	T	T	T	T	T	T	T	T(G)
3	1	646	G	G	**T**	G	G	G	G	G	G	G	G(A)	G	G(AC)
3	1	1000	A	A	**C**	A	A	A	A	A	A(T)	A	A	T(A)	A(T)
3	1	1040	T	T	**G**	T	T	T	T	T	T(A)	T	T	A(T)	T(A)
6	2	385	G	G	G	G	**T**	G	G	G	G	G	G	G	G
6	3	811	C	C(T)	C	C(T)	**A**	C	C	C	C	C	CT	C	C
6	3	842	T	T	T	T	**A**	T(C)	T	T	T	T	T	T	T
6	3	845	G	G	A	A	**T**	A	G	A	A	A	A	A	A
6	3	1336	C	C	C	C(G)	**T**	C	C	C	C	C	C	C	C
7	3	143	T	A(T)	A	T(A)	T	**C**	A	A	T	T	A	A(T)	T
7	2	662	T(G)	G	T	T	T	**A**	T	G	T	T	T	G	T(G)
7	2	682	G	G	G	G	G	**A**	G	G	**A**	**A**	G	G	GT
7	1	830	GA(C)	A(G)	G	AG	A	**T**	G	G	C	C	G	C	C
7	1	856	GTC	T(C)	G	T	T	**A**	T	C	G	G	C	G	G
4 and 8	1	614	C	C	C	**G**	A	C	**G**	C	A	CT	C	C(T)	C
4 and 8	1	626	G	G	G	**C**	T	G	**C**	G	T(G)	GT	G	G	G
4 and 8	1	1123	A	T	T	**G**	A	A	**G**	A	A	A	T	A	A
4 and 8	1	1150	T	A	A	**C**	T	T	**C**	T	T	T	A	T	T
8	1	123	C	C	C	C	C	C	**G(−)**	C	C	C	**T**	C	C
8	1	240	T(A)	T	T	T	T	T	**C**	T	T	T	T	T	T
8	1	238	G	G	G	G	G	G	**T**	G	G	G	**A**	G	G
8	1	286	C	C	C	C	C	C	**T**	C	C	C	C	C	C
8	3	382	A	A	A	A	A	A	**G**	A	A	A	A	A	A
9	2	399	G	G	G	G	G	G	G	**A**	G	G	G	G	G
9	3	858	A(G)	G	G	G	A	G	G	**T**	G	G	G	G	G
9	1	1311	A	A(T)	A	A	A	A	A	**G**	A	A	A	A	A
9	1	1326	T	T	T	T	T	T	T	**C**	T	T	T	T	T
10	1	579	A	A	A	A(−)	G	A	A	A	**T**	G−	A	A	G(A)
10	1	762	T	T	T	T	C	T	T	T	**A**	C	T	T	T
11, 13, 15	2	304	T	T	T	T	T	T	T	T	T	T	T	**A**	T
11, 13, 15	2	734	G	G	G	G	G	G	G	G	G	G	G	**A**	G(T)
11, 13, 15	2	736	C	C	C	C	C	C	C	C	C	C	C	**T**	C(A)
11, 13, 15	1	770	C	C	C	C	C	C	C	C	C	C	C	**T**	C
11, 13, 15	1	809	G	G	G	G	G	G	G	G	G	G	G	**A**	G
11, 13, 15	3	630	A(−)	A	A	A	A	A	A	A	A	A	A	**T(A)**	A
11, 13, 15	1	1357	A(−)	A	A	A	A	A	A	A	A	A	A	**G(A)**	A
11, 13, 15	1	1365	G(−)	G	G	G	G	G	G	G	G	G	G	**A(G)**	G
16, 17, 19	2	166	CT	GCT	C(T)	T	C	T	C	C	C	C	T	CT	**A**
16, 17, 19	1	240	G	G	G	G	G	G	**A**	G	G	G	G	G	**C**
16, 17, 19	1	286	C	C	C	C	C	C	**T**	C	C	C	C	C	**G**
16, 17, 19	2	851	GC	G	G	G	G	G	G	G	G	G	G	G	**T**
16, 17, 19	3	1100	C	C	C	C	C	C	C	C	C	C	C	C	**T**


AF316144 / Pseudoalteromonas issachenkonii / KMM 3549T|OGU=1
AJ417594 / Pseudoalteromonas agarivorans / KMM 255 T|OGU=1
AF043742 / Pseudoalteromonas distincta / KMM 638 T|OGU=1
AF082562 / Pseudoalteromonas elyakovii / KMM 162 T|OGU=1
X82143 / Pseudoalteromonas espejiana / NCIMB 2127 T|OGU=1
X82134 / Pseudoalteromonas atlantica / IAM 12927 T|OGU=1
X82140 / Pseudoalteromonas undina / NCIMB 2128 T|OGU=1
AF529062 / Pseudoalteromonas citrea / CIP 105339T|OGU=1
AF038846 / Pseudoalteromonas gracilis / B9|OGU=1
X98336 / Pseudoalteromonas antarctica / NF3 T|OGU=1
X67024 / Pseudoalteromonas haloplanktis ATCC 14393T|OGU=1
X82146 / Pseudoalteromonas nigrifaciens / NCIMB 8614 T|OGU=1
X82136 / Pseudoalteromonas carrageenovora / ATCC 12662 T|OGU=1
U85855 / Pseudoalteromonas prydzensis / ACAM 620 T|OGU=1
AF475096 / Pseudoalteromonas porphyrae / Q-1-6|OGU=1
X82144 / Pseudoalteromonas luteoviolacea / NCIMB 1893 T|OGU=1
X82147 / Pseudoalteromonas rubra / ATCC 29570 T|OGU=1
X82215 / Pseudoalteromonas piscicida / ATCC 15057T|OGU=1
AF297958 / Pseudoalteromonas flavipulchra / NCIMB 2033T|OGU=1
AF144036 / Pseudoalteromonas maricaloris / KMM 636 T|OGU=1
AF316891 / Pseudoalteromonas ruthenica / KMM 300 T|OGU=1
X82138 / Pseudoalteromonas denitrificans / ATCC 43337T|OGU=1
AF172987 / Pseudoalteromonas ulvae / UL12 T|OGU=1
D89929 / Pseudoalteromonas bacteriolytica / IAM14595 T|OGU=14
Y14697 / Psychromonas antarctica / DSM 10704 T|OGU=6
AB052160 / Psychromonas kaikoae / JT7304|OGU=6
AJ416756 / Psychromonas profunda / 2825 T|OGU=6
AB023378 / Psychromonas marina / 4-22 T|OGU=6
AJ440215 / Idiomarina baltica / OS145 T|OGU=7
AF052741 / Idiomarina zobellii / KMM 231 T|OGU=7
AF052740 / Idiomarina abyssalis / KMM 227T|OGU=7
AF288370 / Idiomarina loihiensis / |OGU=7
AJ294748 / Thalassomonas viridans / DSM 13754 T|OGU=10
U85847 / Colwellia hornerae / ACAM 607 T|OGU=10
U85845 / Colwellia demingiae / ACAM 459 T|OGU=10
U85846 / Colwellia psychrotropica / ACAM 179 T|OGU=10
AY167307 / Colwellia rossensis / ANT9250|OGU=10
AB002630 / Colwellia maris / ABE-1 T|OGU=10
AF001375 / Colwellia psychrerythraea / ATCC 27364T|OGU=10
AJ306890 / Cobetia marina / DSM 4741 T|OGU=19
AF452103 / Pseudomonas cellulosa / NCIMB 10462|OGU=16
AF074383 / Pseudomonas migulae / CIP 105470 T|OGU=17
U01916 / Pseudomonas flavescens / B62 T|OGU=17
AF288360 / Alteromonas alvinellae / HY757 T|OGU=13
Y18228 / Alteromonas macleodii / DSM 6062 T|OGU=13
AF529060 / Alteromonas marina / SW-47|OGU=13
U85854 / Glaciecola pallidula / ACAM 615 T|OGU=11
U85853 / Glaciecola punicea / ACAM 611 T|OGU=15
AJ550470 / Oceanisphaera litoralis / DSM 15406 T|OGU=2
AB019390 / Oceanimonas doudoroffii / ATCC 27123T|OGU=2
AF168367 / Oceanimonas baumannii / ATCC 700832 T|OGU=2
X60416 / Aeromonas schubertii / ATCC 43700 T|OGU=3
AB027005 / Aeromonas salmonicida / NI-441|OGU=3
AF418212 / Aeromonas veronii / 4|OGU=3
S39232 / Aeromonas allosaccharophila / CECT 4199T|OGU=3
X93021 / Ferrimonas balearica / PAT-T|OGU=18
U91546 / Shewanella algae / IAM 14159T|OGU=5
AF005248 / Shewanella amazonensis / SB2B T|OGU=5
AF173966 / Shewanella baltica / KT0246|OGU=5
AF145921 / Shewanella japonica / KMM 3299 T|OGU=5
AY190533 / Shewanella gaetbuli / TF-27|OGU=5
X81623 / Shewanella putrefaciens / LMG 26268T|OGU=5
AF033028 / Shewanella saccharophilus / GC-29|OGU=5
AF005251 / Shewanella oneidensis / MR-1 T|OGU=5
U85903 / Shewanella frigidimarina / ACAM 591 T|OGU=5
AJ300834 / Shewanella livingstonensis / LMG 19866 T|OGU=5
AJ457093 / Shewanella denitrificans / OS220|OGU=5
AY170366 / Shewanella waksmani / KMM 3823 T|OGU=5
AF420312 / Shewanella fidelis / KMM 3582 T|OGU=5
X82132 / Shewanella hanedai / CIP 103207 T|OGU=5
AF003549 / Shewanella woodyi / MS32 T|OGU=5
X82131 / Shewanella benthica / ATCC 43992 T|OGU=5
D21225 / Shewanella violacea / DSS12 T|OGU=5
U85907 / Shewanella gelidimarina / ACAM 456 T|OGU=5
X82142 / Moritella marina / NCIMB 1144 T|OGU=9
AB038033 / Moritella marina / ATCC 15381 T|OGU=9
AJ132226 / Moritella viscosa / NVI 88 478 T|OGU=9
D21224 / Moritella japonica / DSK1T|OGU=9
AB008797 / Moritella yayanosii / DB21MT-T|OGU=9
AF057016 / Salinivibrio vallismortis / DSM 8285|OGU=8
M98446 / Vibrio aspartigenicus / GSP-1|OGU=8
X74699 / Salinivibrio costicola / ATCC 35508 T|OGU=8
D25307 / Photobacterium angustum / ATCC 25915T|OGU=4
AB016982 / Photobacterium indicum / MBIC 3157|OGU=4
AB003191 / Photobacterium profundum / SS9|OGU=4
AB000278 / Vibrio iliopiscarius / ATCC 51760 T|OGU=4
Z19107 / Photobacterium phosphoreum / Og61|OGU=4
X74702 / Vibrio fischeri / ATCC774 T|OGU=4
AJ437616 / Vibrio logei / NCIMB 2252|OGU=4
X70643 / Vibrio salmonicida / NCMB 2262 T|OGU=4
AJ132227 / Vibrio wodanis / NVI 88 441 T|OGU=4
AJ310647 / Vibrio agarivorans / CECT 5085 T|OGU=4
AB013297 / Vibrio rumoiensis / S-1 T|OGU=4
AF124055 / Vibrio aerogenes / ATCC 700797T|OGU=4
X74723 / Vibrio proteolyticus / ATCC 15338T|OGU=4
X76333 / Vibrio vulnificus / ATCC 27562 T|OGU=4
X74716 / Vibrio nereis / ATCC 25917 T|OGU=4
X74725 / Vibrio tubiashii / ATCC 19109 T|OGU=4
AJ440004 / Vibrio corallilyticus / LMG 21349 T|OGU=4
X74704 / Vibrio fumissii / ATCC 350116 T|OGU=4
X76335 / Vibrio fluvialis / NCTC 11327 T|OGU=4
AJ550855 / Allomonas enterica / ATCC 35843 T|OGU=4
X99761 / Vibrio mytili / |OGU=4
X99762 / Vibrio diabolicus / HE800T|OGU=4
X74690 / Vibrio alginolyticus / ATCC 17749T|OGU=4
X74722 / Listonella pelagia / ATCC 25916 T|OGU=4
X74714 / Vibrio natriegens / ATCC 14048 T|OGU=4
X74692 / Vibrio campbellii / ATCC 25920 T|OGU=4
X74706 / Vibrio harveyi / ATCC 14126 T|OGU=4
X74693 / Vibrio carchariae / ATCC 35084 T|OGU=4
AF388387 / Vibrio parahaemolyticus / ATCC 17802 T|OGU=4
AJ421444 / Vibrio penaeicida / DSM 14398T|OGU=4
AB000390 / Vibrio halioticoli / IAM14596 T|OGU=4
AY155583 / Vibrio superstes / G3-11|OGU=4
U80200 / Pectobacterium chrysanthemi / ATCC 11663 T|OGU=12
Z49829 / Yersinia enterocolitica / ER 26036 92|OGU=12
AF130971 / Pantoea cedenensis / A34|OGU=12
AF130885 / Pantoea toletana / A4|OGU=12
AF025373 / Citrobacter werkmanii / CDC 0876 58T|OGU=12
99% +x
96% +
100%+x
94% +
100%+x
100%+x
I
85% x
100%+x
II
46% +x
100%+x
99%+x
III
100% +x
82% x
IV
100% +
100%+x
76%+x
100%+x
83%+x
100%+x
82%+x
100%+x
95%x
100%x
99%+x
68%+
100%+x
V
94% +x
62%+x
100%+x

Housekeeping genes

Other housekeeping genes such as *gyrB*, *rpoB* and *recA* would have been useful, but the sequences of too many genera are still missing (*gyrB*, four entries for proteobacteria; *rpoB*, 352 entries for '*Gammaproteobacteria*' and, for the *Alteromonadales*, only five sequences of *Shewanella*, two complete, three partial 100 nt; *recA*, 546 sequences for '*Gammaproteobacteria*', for the *Alteromonadales*, no sequence, for vibrios, 216 entries). Finally, the 16S–23S intergenic spacer region is much too divergent for use in phylogenetic analyses at the family level.

Signature nucleotide positions

The use of signature nucleotide positions. Signature nucleotides are nucleotide residues that are found explicitly in all currently described species of a proposed taxon and not in other taxa. Signatures sites may be particularly strong when they consist of compensatory mutations involved in maintaining the secondary structure of the molecule (see Methods). Taking into account signature sites is only an improvement in phylogenetic analyses, as no phylogenetic method can yet take into account that different positions should be weighted differently according to the selective pressure that applies at every position. It is possible to use weights, but we do not yet know how to define what weight to apply according to the position in the structure.

Taxon identification with signature positions. Confirmation of the branching order of the *Alteromonas*-like bacteria was sought by searching for signature nucleotide positions (Table 1). The signature patterns must be considered tentative since, with a significant increase in the number of sequences in any phylogenetic lineage, the number of signature nucleotides may decrease because of possible new mutations (and/or sequencing errors overlooked).

Careful examination of Table 1 suggested that some mutations were compensatory (for example, a G–C pair instead of an A–T pair). We then added the *E. coli* sequence in our alignment and mapped all of the signatures on the secondary structure of the 16S rRNA molecule. Since all *Alteromonas*-like bacteria belong to the '*Gammaproteobacteria*' and hence are related to *E. coli*, there is little variation in the secondary structure, except for the variable parts of helices indicated with blue dashed lines on Fig. 2. This analysis showed that signature positions can be divided into three classes: (i) single compensatory mutations, for example a G–C pair mutating to G–U, (ii) double compensatory mutations (transition), for example A–T to G–C, and finally (iii) mutations affecting nucleotides not paired in the secondary structure (indicated in Table 1). Observations of single or double compensatory mutations are rather strong arguments that the signatures observed are not the result of errors in sequences. The last type of mutations is quite interesting: since these mutations are conserved for a large cluster of species, it means that there is rather strong selective pressure on these nucleotides, suggesting that they might be part of interactions involving the structures of the rRNA at the tertiary or quaternary level (within 16S rRNA or between 16S and 23S rRNA).

Simple linkage sequence aggregations

Grouping sequences in OGUs. Finally, we investigated a classical classification system, i.e. grouping together sequences that share a percentage of similarity above a given threshold. The main problem is to decide the cut-off level for aggregation. We found, as expected, that the sequences studied were distributed in decreasing numbers of OGUs as the cut-off percentage chosen for aggregation decreased (Supplementary Fig. E). Interestingly, a plateau was observed for 93–94 % 16S rRNA gene sequence similarity (see Methods). We then used 93 % similarity to label OGUs in the tree of Fig. 1.

Discrimination of genera. The results revealed that there was a good correlation at the similarity level between genera, signature sites and OGUs, though there were a few exceptions. One exception was the genus *Pseudoalteromonas* in its current state, but this problem was solved as proposed below, by creating a separate genus, *Algicola*, for [*Pseudoalteromonas*] *bacteriolytica*. A second exception was the genus *Glaciecola*. Bacteria of this genus shared many signature nucleotides with *Alteromonas* species. This finding might be a strong argument for the unification of these two genera. Indeed, these bacteria are close genetically (similar G+C content of the DNA) and share many phenotypic and chemotaxonomic characteristics. They were, however, distributed in different OGUs, which is an argument to keep distinct genera. *Vibrio* and *Photobacterium* were grouped in a single OGU (with the exception of '*Vibrio aspartigenicus*'). The taxonomic status of these genera seems far from clear, since detailed phylogenetic analyses (not shown) suggest that the two genera are in fact intermingled; a possible solution would be to reduce them to a single genus. For these taxa, data on housekeeping genes are clearly required. *Oceanimonas* and *Oceanisphaera* were in a single OGU, but we were not able to find signature nucleotides for this cluster. A close examination of the only sequences available for two of the three species suggested the presence of obvious sequencing

Fig. 1. Phylogenetic consensus tree indicating the phylogenetic position of the *Alteromonas*-like and related bacterial taxa. The unrooted tree shown is the result of an NJ bootstrap analysis (1000 replications) using 16S rRNA gene sequences. Branches that were retrieved also by MP (three most parsimonious trees) and ML (ln = −3506) are indicated respectively by + and x ($P<0{\cdot}01$). Major clusters discussed in this paper (OGUs and accession numbers indicated) are indicated by vertical bars that bear the same colours as those used to indicate the mutations on the secondary structure (Fig. 2).

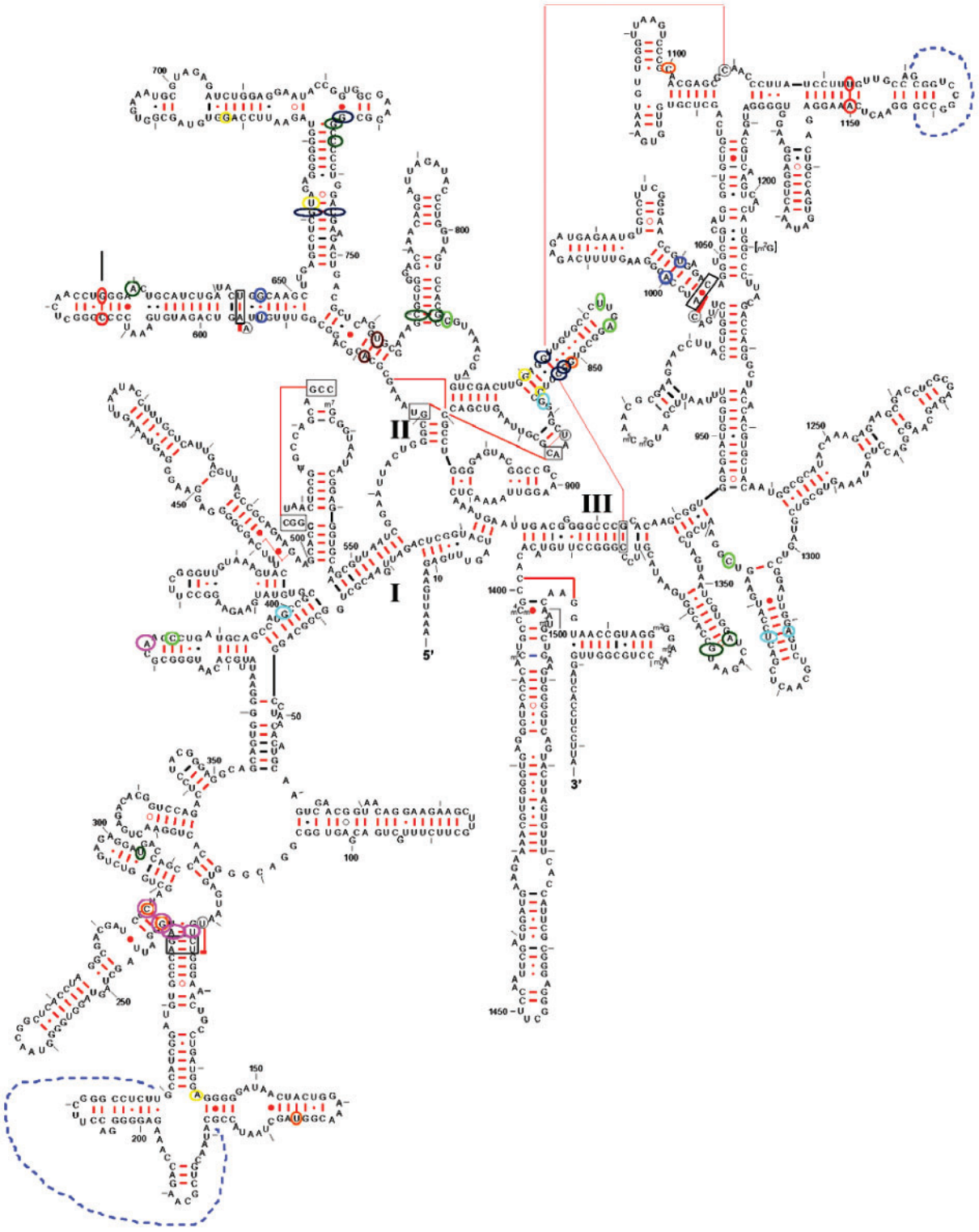


Fig. 2. Locations of characteristic signature nucleotides of *Alteromonas*-like and related taxa localized in the secondary structure of the small-subunit rRNA (*E. coli*). Nucleotides are colour-coded as in Fig. 1, which allows the visualization of each cluster where mutations are localized. Blue dashed lines: parts of the molecule where the secondary structure may change in the various taxa and that were not assessed for the presence of signature sites. The original cartoon was taken from http://www.rna.icmb.utexas.edu (accession no. J01695. November 1999; cosmetic changes July 2001).

errors (a different nucleotide at a position otherwise conserved in all other sequences). The availability of more and better sequences should solve this problem. Finally, *Thalassomonas* and *Colwellia* were also placed in a single OGU. Analysis of sequence signatures suggested splitting the genus *Colwellia* into two genera. A more detailed analysis of this clade is required.

Formal rules for genus identification

So far, there is no formal rule for delimitation of a taxon above the species level. The general position is to define a genus when there is a robust branch that clearly delineates a clade in phylogeny and when these organisms present distinct shared phenotypes. This is often problematic when members of a group have adapted to very different ecological niches, resulting in divergent phenotypes. Our analyses in terms of aggregative OGUs sharing a minimal level of 16S rRNA gene similarity, comparison with the phylogeny and sequence signatures suggest that it might be possible to use useful criteria for taxon delineation above the species level; this would extend the present criteria used at the species level: percentage of DNA–DNA association and 97 % 16S rRNA gene sequence similarity (Stackebrandt & Goebel, 1994). Aggregative clustering at the 93 % similarity level and the presence of site signatures may be used as a general criterion to help to decide at which level in a tree a genus can be defined. If such an approach was shown to be possible for the genera analysed in this study, studies of different groups (e.g. *Actinobacteria*, *Cyanobacteria*, *Archaea*) are required before we can make a definitive proposal. Presently, we were not able to use lower percentages of aggregation to try to define families, since the OGUs obtained were largely inconsistent with the phylogeny. A possible reason might be that domains that are too divergent for that level of taxonomy exist in the complete rRNA sequences. Restriction of the analysis to conserved domains might be a solution. We are presently investigating the use of more sophisticated clustering algorithms, restricting to more conserved domains as well as studying different groups of bacteria in order to propose a generalization of the procedure.

In conclusion, the following monophyletic groups can be distinguished.

Group I, including *Pseudoalteromonas* species and [*Pseudoalteromonas*] *bacteriolytica*

The *Pseudoalteromonas* cluster, encompassing more than 30 species, was relatively heterogeneous, with interstrain 16S rRNA gene sequence similarity values ranging from 90 to 99·9 % (see Supplementary Table A). The results obtained are consistent with previous detailed phylogenetic analysis (Gauthier *et al.*, 1995) and confirmed that species of the genus consisted of several monophyletic taxa. One of them comprises a closely related group of so-called non-pigmented species (currently includes 15 species, including the type strain of the genus, *Pseudoalteromonas haloplanktis*; *Pseudoalteromonas nigrifaciens* and *Pseudoalteromonas distincta* can produce melanin-like pigments depending on culture medium) with high interstrain similarity values of 98–99·9 %. Other species in the genus are pigmented, synthesizing a variety of pigments (prodigiosin-like, carotenoids and some other pigments), and could be split into six clusters: (i) *Pseudoalteromonas citrea* and *Pseudoalteromonas aurantica*, (ii) *Pseudoalteromonas ruthenica*, (iii) *Pseudoalteromonas rubra*, *Pseudoalteromonas luteoviolacea*, *Pseudoalteromonas peptidolytica*, *Pseudoalteromonas piscicida*, *Pseudoalteromonas flavipulchra* and *Pseudoalteromonas mariscaloris*, (iv) *Pseudoalteromonas tunicata* and *Pseudoalteromonas ulvae*, (v) *Pseudoalteromonas denitrificans* and (vi) [*Pseudoalteromonas*] *bacteriolytica* (see detailed tree; Supplementary Fig. B). This last species branched deeply and was a sister species to all other species. The deep branching (low similarity levels for nucleotides of 16S rRNA down to 90·3 %), the lack of sequence signature, the lack of association with other species of the genus, low DNA–DNA hybridization values (3–5 %) and some characteristic phenotypic traits (bacteriolytic activity, requirement for organic growth factors, different pattern of carbohydrate utilization) indicated that this bacterium should be placed in a separate genus. Therefore, we propose to create a new family, *Pseudoalteromonadaceae* fam. nov., which comprises two genera, *Pseudoalteromonas* and *Algicola* gen. nov., which contains *Algicola bacteriolytica* comb. nov. as its type species.

Group II, including *Idiomarina* species, *Colwellia* species and *Thalassomonas viridans*

Group II appeared as a clade that comprised two subclades strongly supported by bootstrap (Fig. 1 and Supplementary Fig. D): one cluster including all *Colwellia* species and *Thalassomonas viridans* and a second cluster including *Idiomarina* species. Species of the genus *Colwellia* failed to share common signature nucleotides, except when the deeply branched sequence of *Colwellia hornerae* was removed from the analysis. It is possible that some errors in sequences might be responsible for the failure to find characteristic signatures; on the other hand, the deep branching of *C. hornerae* and peculiar characteristic phenotypic traits (sensitivity to vibriostatic agent O/129, the lack of sequence signature, the lack of the ability to produce chitinase, different pattern of carbohydrate utilization) and distinct cellular fatty acid composition, e.g. significantly high proportions of 15 : 1(n-8), 15 : 0 and i16 : 0 fatty acids (Bowman *et al.*, 1998a), are indications that this species may need to be recognized as representing a separate genus. A detailed study of this cluster is necessary.

Because of the weak bootstrap percentage uniting *Idiomarina* with the other genera, it should be retained as a separate taxon. In this context, we propose to create the new families *Colwelliaceae* fam. nov., restricted to bacteria of the genera *Colwellia* and *Thalassomonas*, and *Idiomarinaceae* fam. nov. Further sequences for novel species or

different genes may help to improve certainty in this part of the tree.

Group III, including *Alteromonas* and *Glaciecola* species

This clade included the genus *Alteromonas* and two species of *Glaciecola*. We therefore propose to restrict the recently proposed family *Alteromonadaceae* (Ivanova & Mikhailov, 2001) to only these two genera, since the taxonomic placement of bacteria of the genera *Pseudoalteromonas*, *Idiomarina* and *Colwellia* within the family appears not to be appropriate (Fig. 1 and Supplementary Fig. C).

Group IV, including *Aeromonas*, *Oceanisphaera* and *Oceanimonas* species

Oceanimonas and *Oceanisphaera* species formed a loosely supported clade (only two methods) and, with *Aeromonas* species, a loosely supported clade was also revealed (two methods also, although different ones). For the reasons mentioned above, taxonomic affiliation on the family level remains unclear and the recognition of a family should await more sequences.

Group V, including *Vibrio*, *Salinivibrio* and *Photobacterium* species

These genera formed a robust cluster that constitutes the family *Vibrionaceae*. Importantly, it included the sequences of *Allomonas enterica* and *Hyphomicrobium indicum*; the grouping of the genus *Allomonas* (and also the genus *Listonella*) within the genus *Vibrio* still awaits nomenclatural clarification. Since these sequences could result from sequencing of contaminants, one should await the appearance of at least a second sequence before adjusting their taxonomy (as also suggested by Thompson *et al.*, 2003).

Isolated genera

Bacteria of the four genera *Shewanella*, *Moritella*, *Psychromonas* and *Ferrimonas* (Fig. 1) did not form a monophyletic clade with other genera included in this study, at least when using 16S rRNA gene sequences as support of phylogenetic information. The *Shewanella* cluster, encompassing 22 species, was rather heterogeneous, with 16S rRNA gene sequence similarity values ranging from 93 to 99·9 %. Notably, a single signature nucleotide, 858 (C), was identified; this small number may result from errors in some sequences. Our results confirmed previous observations (Venkateswaran *et al.*, 1999; Nogi & Kato, 2002) that a few monophyletic clusters are constantly recovered for *Shewanella* species.

Bacteria of the genera *Moritella*, *Psychromonas* and *Ferrimonas* clustered separately at the family level (supported by specific signature nucleotides; Table 1) and could not be grouped with any other taxa.

Thus, phylogenetic classification performed in this study was consistent with phenotypic and polyphasic classifications and has led to the grouping of these bacteria into several taxa on the family level as follows: *Alteromonadaceae* emend., including genera *Alteromonas* and *Glaciecola*, *Pseudoalteromonadaceae* fam. nov., including genera *Pseudoalteromonas* and *Algicola* gen. nov., *Colwelliaceae* fam. nov., comprising *Colwellia* and *Thalassomonas*, and the monogeneric families *Idiomarinaceae* fam. nov., *Ferrimonadaceae* fam. nov., *Shewanellaceae* fam. nov., *Moritellaceae* fam. nov. and *Psychromonadaceae* fam. nov.

The sources, habitats and differential characteristics of the proposed taxa are summarized in Table 2. Bacteria of the family *Alteromonadaceae* are aerobic, slightly halophilic organisms, distinct in the inability to hydrolyse chitin and agar, but able to utilize a range of carbohydrates. Bacteria of these genera can be distinguished by pigmented colonies, G+C content of their DNA and psychrophily. The numerous species of the family *Pseudoalteromonadaceae* are diverse in their phenotypic traits and difficult even in tentative classification. However, bacteria of the newly proposed genus *Algicola* can be easily differentiated from other pseudoalteromonads by the lack (or weak activity) of catalase, limited temperatures for growth (from 15 to 35 °C) and presence of bacteriolytic activity (Sawabe *et al.*, 1998). Though the bacteria of the two families *Alteromonadaceae* and *Pseudoalteromonadaceae* have similar cellular fatty acid compositions, the different ratio of major cellular fatty acids allows separation at the genus level (Svetashev *et al.*, 1995; Ivanova *et al.*, 2000b). Bacteria of the family *Colwelliaceae* are both aerobic and facultatively anaerobic, obligatorily marine organisms that require sodium ions for growth and include pigmented and non-pigmented species; many of those hydrolyse chitin, gelatin, starch and Tween 80. Bacteria of the genus *Colwellia* are different from those of *Thalassomonas* in their ability to reduce nitrate to nitrite and psychrophily. Bacteria of the family *Idiomarinaceae* differ from colwellias and thalassomonads by the ability to tolerate high concentrations of NaCl and limited ability to utilize carbohydrates. The bacteria of the genera *Colwellia*, *Thalassomonas* and *Idiomarina* have characteristic patterns of cellular fatty acids (Bowman *et al.*, 1997; Russell & Nichols, 1999; Macián *et al.*, 2001). Bacteria of the families *Shewanellaceae* and *Moritellaceae* consist of both aerobic and facultatively anaerobic organisms that require sea water or sodium ions for growth and can be distinguished by halophilicity and by the ability to synthesize either eicosapentaenoic (20 : 5ω3) or docosahexaenoic (22 : 6ω3) acid, respectively.

Emended description of *Alteromonadaceae* Ivanova and Mikhailov 2001

Alteromonadaceae (Al.te.ro.mo.na.da′ce.ae. N.L. fem. n. *Alteromonas* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Alteromonadaceae* the *Alteromonas* family).

Gram-negative, rod-shaped bacteria. Motile. Do not form

Table 2. Differential characteristics of marine gammaproteobacteria of the families *Alteromonadaceae*, *Pseudoalteromonadaceae*, *Colwelliaceae*, *Idiomarinaceae*, *Shewanellaceae*, *Moritellaceae*, *Ferrimonadaceae* and *Psychromonadaceae*

ND, No data available; W, weak reaction; V, reaction is different depending on strain. All taxa show polar flagellation.

Characteristic	*Alteromonadaceae*		*Pseudoalteromonadaceae*		*Colwelliaceae*		*Idiomarinaceae*	*Oceanimonas* and *Oceanisphaera*	*Shewanellaceae*	*Moritellaceae*	*Ferrimonadaceae*	*Psychromonadaceae*
	Alteromonas	*Glaciecola*	*Pseudoalteromonas*	*Algicola*	*Colwellia*	*Thalassomonas*						
Pigmentation	−	+	−/+	+	+/−	+	−	−	−/+*	−	+*	−
Flagellation:												
Bipolar	−	−	−/+	−	−	−	−	−	−	−	−	−
Lateral	−	−	−/+	−	−	−	−	−	−	−	−	−
Outer coat	−	−	−	−	−	−	+	−	−	−	−	−
Metabolism†	A	A	A‡	A	A/F	A	A	A	A/F	A/F	F	AN
Growth without sea water salts	−	−	−	−	−	−	−	−	+	−	−	+
Halotolerance (% NaCl)	6	8	15	6	20	3	15	7	10	4	7·5	4
Growth at:												
4 °C	−	+	+	−	+	−	+	−	+	+	−	+
37 °C	+	−	+	−	−	+	−	+	−	−	+	−
42 °C	−	−	−	−	−	−	−	−	−	−	+	−
Hydrolysis of:												
Chitin	−	−	V	−	+	ND	V	ND	V	ND	ND	ND
Agar	−	−	V	−	−	ND	−	−	V	ND	ND	ND
Starch	+	V	V	+	V	+	−	ND	V	−	−	+
Gelatin	+	+	+	+	V	+	+	−	V	+	ND	ND
Utilization:												
D-Glucose	+	+	+	+	+	+	−	−	+	V	ND	+
D-Fructose	+	−	V	+	V	+	−	ND	−	V	ND	+
D-Mannose	V	−	V	+	V	ND	−	−	V	V	ND	−
L-Rhamnose	+	+	V	ND	−	W	−	−	V	−	ND	−
Sucrose	+	+	V	+	V	+	−	−	−	−	ND	ND
Cellobiose	+	+	V	−	V	ND	−	−	V	V	ND	+
Lactose	+	+	V	−	V	+	−	−	V	−	ND	+
Glycerol	+	V	V	−	V	−	−	V	−	V	ND	−
Major fatty acids	16:0, 16:1ω7, 18:1ω7	16:0, 16:1ω7, 18:1ω7	16:0, 16:1ω7, 18:1ω7	16:0, 16:1ω7, 18:1ω7	16:1ω7, 16:0, 22:6ω3	15:0, 16:0, 17:1ω8	i15:0, i17:0, 18:1ω7	16:0, 16:1*cis*, 18:1*cis*	i13:0, 14:0, 16:0, 16:1, 20:5ω3	14:0, 16:0, 16:1, 22:6ω3	i15:0, 17:1ω9, 17:0	ND
Known habitats§	S	Si	S, Inv, A	A	Si	Inv	S	Em	S, Inv	S, B, F	B	B
G+C content of DNA (mol%)	44-48	40-46	37-50	44-46	35-46	48·4	48-50	54	39-52	44-45	54·0	42·8

*Colonies on TSI medium produce a black iron precipitate.

†A, Aerobic; AN, anaerobic; F, facultatively anaerobic.

‡According to the original description, *Pseudoalteromonas tunicata* is facultatively anaerobic.

§A, Algae; B, benthic sediments; Em, estuarine mud; F, fish; Inv, invertebrates; S, sea water; Si, sea ice.

endospores or microcysts. Chemo-organotrophs. Oxygen is used as the electron acceptor. Aerobic or facultatively anaerobic. Usually do not denitrify. Arginine dihydrolase is absent. Require Na^+ ions for growth. In most species, the major isoprenoid quinone is Q8. The major fatty acids are 16 : 0, 16 : 11ω7 and 16 : 1ω7. Members of the family have been isolated from coastal, open and deep-sea waters and invertebrates from marine environments. The family is a member of the '*Gammaproteobacteria*' with the following nucleotide sequence characteristics: 304 (A), 734 (A), 736 (T), 770 (T), 809 (A). The family comprises the type genus *Alteromonas* Baumann *et al.* 1972 emend. Gauthier *et al.* 1995 and the genus *Glaciecola* Bowman *et al.* 1998.

Description of *Pseudoalteromonadaceae* fam. nov.

Pseudoalteromonadaceae (Pseu.do.al.te.ro.mo.na.da′ce.ae. N.L. fem. n. *Pseudoalteromonas* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Pseudoalteromonadaceae* the *Pseudoalteromonas* family).

Gram-negative, rod-shaped bacteria. Motile by means one or several flagella (sometimes coated); some species have lateral or bipolar flagella. Some species produce capsules. Chemo-organotrophs. Require Na^+ ions for growth; some strains are capable of growing in media containing 15 % NaCl. Aerobic or facultatively anaerobic. Usually do not denitrify. Arginine dihydrolase is absent. In most species, the major isoprenoid quinone is Q8. The major fatty acids are 16 : 0, 16 : 1ω7 and 18 : 1ω7. Members of the family have been isolated from coastal, open and deep-sea waters, sediments, marine invertebrates, fish and algae from marine environments. The family is a member of the '*Gammaproteobacteria*' with the following nucleotide sequence characteristics: 733 (A), 744 (T), 833 (C), 852 (T), 853 (T). The family comprises the type genus *Pseudoalteromonas* Gauthier *et al.* 1995 and the genus *Algicola* gen. nov.

Description of *Algicola* gen. nov.

Algicola (Al.gi.co′la. L. n. *alga -ae* a seaweed; L. suff. *-cola* from L. n. *incola* an inhabitant, dweller; N.L. fem. n. *Algicola* inhabitant of algae).

Gram-negative, strictly aerobic, chemo-organotrophic organisms. Motile by means of a single flagellum. Oxidase-positive. Requires sea water or addition of marine salts and organic factors for growth. Mesophilic. Have bacteriolytic activity. The genus is affiliated to the '*Gammaproteobacteria*' and contains one species, *Algicola bacteriolytica*, which is the type species.

Description of *Algicola bacteriolytica* (Sawabe *et al.* 1998) comb. nov.

Basonym: *Pseudoalteromonas bacteriolytica* Sawabe *et al.* 1998.

Description is identical to that given by Sawabe *et al.* (1998). The DNA G+C content of the type strain is 46·0 mol%. The type strain is IAM 14595^T (=ATCC 700679^T=CIP 105725^T).

Description of *Shewanellaceae* fam. nov.

Shewanellaceae (She.wa.nel.la′ce.ae. N.L. fem. n. *Shewanella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Shewanellaceae* the *Shewanella* family).

Gram-negative, rod-shaped bacteria. Motile. Do not form endospores or microcysts. Chemo-organotrophs. Aerobic or facultatively anaerobic. Able to reduce nitrate to nitrite and grow anaerobically by reducing trimethylamine *N*-oxide and ferric compounds. Some species do not require Na^+ ions for growth. In most species, the major isoprenoid quinones are Q7, Q8 and MK7. The major fatty acids are 14 : 0, 16 : 1ω7, 16 : 0 and 17 : 1ω6. Most species produce PUFA. Members of the family have been isolated from coastal, open and deep-sea waters and invertebrates from marine environments. The family is a member of the '*Gammaproteobacteria*'. The type genus is *Shewanella* MacDonell and Colwell 1985.

Description of *Moritellaceae* fam. nov.

Moritellaceae (Mo.ri.tel.la′ce.ae. N.L. fem. n. *Moritella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Moritellaceae* the *Moritella* family).

Gram-negative, curved or straight rod-shaped bacteria. Motile. Do not form endospores or microcysts. Chemo-organotrophs. Aerobic or facultatively anaerobic. Require Na^+ ions for growth. Usually do not denitrify. Arginine dihydrolase is absent. Require Na^+ ions for growth. In most species, the major isoprenoid quinones are Q7 and Q8. The major fatty acids are i13 : 0, 14 : 0, 14 : 1, 16 : 0 and 16 : 1. Most species produce PUFA. Members of the family have been isolated from deep-sea waters from marine environments. The family is a member of the '*Gammaproteobacteria*' with the following nucleotide sequence characteristics: 399(A), 858(T), 1311 (G), 1326 (C). The type and only genus is *Moritella* Urakawa *et al.* 1998.

Description of *Idiomarinaceae* fam. nov.

Idiomarinaceae (Idio.ma.ri.na′ce.ae. N.L. fem. n. *Idiomarina* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Idiomarinaceae* the *Idiomarina* family).

Gram-negative, rod-shaped bacteria. Motile. Do not form endospores or microcysts. Chemo-organotrophs. Oxygen is used as the electron acceptor. Aerobic or facultatively anaerobic. Usually do not denitrify. Arginine dihydrolase is absent. Require Na^+ ions for growth. The major fatty acids are i15 : 0 and i17 : 0. Members of the family have been isolated from open and deep-sea waters. The family is a member of the '*Gammaproteobacteria*' with the following nucleotide sequence characteristics: 143 (C), 662 (A), 682

(A), 830 (T), 856 (A). The type and only genus is *Idiomarina* Ivanova *et al.* 2000.

Description of *Colwelliaceae* fam. nov.

Colwelliaceae (Col.wel.li.a′ce.ae. N.L. fem. n. *Colwellia* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Colwelliaceae* the *Colwellia* family).

Gram-negative, curved rod-shaped bacteria. Motile. Some species are non-motile. Do not form endospores or microcysts. Require Na^+ ions for growth. Chemo-organotrophs. Facultatively anaerobic. The major fatty acids are 15 : 1ω8, 15 : 0, i16 : 0, 16 : 1ω7 and 16 : 0. Produce PUFA. The family is a member of the '*Gammaproteobacteria*' with the following nucleotide sequence characteristics: 579 (T), 762 (A). Genera belonging to the family are the type genus *Colwellia* Deming *et al.* 1988 and the genus *Thalassomonas* Macián *et al.* 2001.

Description of *Ferrimonadaceae* fam. nov.

Ferrimonadaceae (Fer.ri.mo.na.da′ce.ae. N.L. fem. n. *Ferrimonas* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Ferrimonadaceae* the *Ferrimonas* family).

Gram-negative, rod-shaped bacteria. Motile. Do not form endospores or microcysts. Chemo-organotrophs. Facultatively anaerobic. Nitrate is reduced to nitrite. Require Na^+ ions for growth. The major fatty acids are i15 : 0, 16 : 1ω9 and 17 : 1ω9. The family is a member of the '*Gammaproteobacteria*'. The type genus is *Ferrimonas* Rosselló-Mora *et al.* 1995.

Description of *Psychromonadaceae* fam. nov.

Psychromonadaceae (Psy.chro.mo.na.da′ce.ae. N.L. fem. n. *Psychromonas* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Psychromonadaceae* the *Psychromonas* family).

Gram-negative, rod- to oval-shaped bacteria. Motile. Do not form endospores or microcysts. Chemo-organotrophs. Aerotolerant anaerobes. Some species do not require Na^+ ions for growth. Arginine dihydrolase is absent. In most species, the major isoprenoid quinone is Q8. The major fatty acids are 16 : 0 and 16 : 1ω7. Members of the family have been isolated from coastal, open and deep-sea waters and invertebrates from marine environments. The family is a member of the '*Gammaproteobacteria*' with the following nucleotide sequence characteristics: 385 (T), 811 (A), 842 (A), 845 (T), 1336 (T). The type and only genus is *Psychromonas* Mountfort *et al.* 1998.

Description of *Photobacterium indicum* (Johnson and Weisrock 1969) comb. nov.

Basonym: *Hyphomicrobium indicum* Johnson and Weisrock 1969.

The description is identical to that given by Johnson & Weisrock (1969). The type strain is MBIC 3157^T [= ATCC 19614^T = DSM 5151^T = IFO (now NBRC) 14233^T].

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