NOTE

Reclassification of *Alteromonas distincta* Romanenko *et al.* 1995 as *Pseudoalteromonas distincta* comb. nov.

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The 16S rRNA gene of *Alteromonas distincta* KMM 638^T was amplified, cloned and sequenced. The nucleotide sequence was aligned with sequences of representative strains of *Alteromonas*, *Moritella*, *Pseudoalteromonas* and *Shewanella*. Results of phylogenetic analysis, using neighbour-joining and Fitch–Margoliash methods, clearly indicated that this species should be assigned to the genus *Pseudoalteromonas*. On the basis of polyphasic data obtained from previous work and this study, it is proposed that the species *Alteromonas distincta* be reclassified as *Pseudoalteromonas distincta* comb. nov. with type strain KMM 638^T (= ATCC 700518^T).

Keywords: reclassification of *Pseudoalteromonas distincta*, *Alteromonas distincta*

Marine *Pseudomonas*-like bacteria comprise several related genera, including *Alteromonas* (Baumann & Baumann, 1981; Baumann *et al.*, 1984), *Marinomonas* (Gauthier & Breittmayer, 1992), *Pseudoalteromonas* (Gauthier *et al.*, 1995), *Moritella* (Gauthier *et al.*, 1995), *Marinobacter* (Gauthier *et al.*, 1992), *Psychrobacter* (Bowman *et al.*, 1996), *Colwellia* (Deming *et al.*, 1988), *Shewanella* (MacDonnell & Colwell, 1985) and *Halomonas* (Dobson & Franzmann, 1996). Classification of these aerobic heterotrophic micro-organisms from the marine environment remains difficult and time-consuming due to their phenotypic diversity and absence of robust chemotaxonomic markers. Fortunately, phylogenetic information, based on 16S rRNA gene sequences, is useful in classifying and identifying micro-organisms belonging to previously poorly defined taxa.

In 1995, Romanenko and colleagues created a new species, *Alteromonas distincta*, into which was placed a bacterium isolated from a marine sponge collected at a depth of 350 m near the Komandorskie Islands, Russia. Using phenotypic and genotypic criteria, these investigators were able to separate *A. distincta* from all validly described species of the genus *Alteromonas*. In an independent phylogenetic study based on 16S rRNA sequences, Gauthier *et al.* (1995) proposed that members of the genus *Alteromonas* be divided into two groups: *Alteromonas*, including the type species *Alteromonas macleodii*, and a cluster of species corresponding to the '*Alteromonas haloplanktis*' cluster of De Vos *et al.* (1989), for which the new genus *Pseudoalteromonas* was proposed. However, the taxonomic position of *A. distincta* was not clear, since it was not included in the study of Gauthier *et al.* (1995). The present study was undertaken to determine the appropriate genus assignment for the species.

A. distincta KMM 638^T (= ATCC 700518^T) (the species is represented by a single strain) was grown at 25 °C on Marine agar 2216 (Difco Laboratories) and stored at –80 °C in Marine broth (Difco) supple-

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The GenBank accession number for the 16S rRNA gene sequence of *Pseudoalteromonas distincta* KMM 638^T is AF043742.

mented with 30 % (v/v) glycerol. Genomic DNA was extracted employing the method of Gauthier *et al.* (1995). PCR, cloning and sequencing of the 16S rRNA gene were carried out using the *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an Applied Biosystems 373A DNA sequencer, as described previously by Chun & Goodfellow (1995). The resultant 16S rRNA gene sequence was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were constructed using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The PHYLIP package (Felsenstein, 1993) was used for all analyses. The resultant unrooted tree topology was evaluated in bootstrap analyses (Felsenstein, 1985), using the neighbour-joining method based on 1000 resamplings.

A 16S rRNA gene sequence (1451 nt) was obtained for the type strain of *A. distincta*. From the unrooted evolutionary tree shown in Fig. 1, it is concluded that *A. distincta* belongs to the genus *Pseudoalteromonas*. The relationship is based on data from different tree-making algorithms and the 100 % bootstrap value. Furthermore, the tree is very similar to that of Gauthier *et al.* (1995). *A. distincta* was recovered within a monophyletic clade, *Pseudoalteromonas*, containing the non-pigmented species, *Pseudoalteromonas antarctica*, *Pseudoalteromonas atlantica*, *Pseudoalteromonas carrageenovora*, *Pseudoalteromonas espejiana*, *Pseudoalteromonas haloplanktis* subsp. *haloplanktis*, *Pseudoalteromonas haloplanktis* subsp. *tetraodonis*, *Pseudoalteromonas nigrifaciens* and *Pseudoalteromonas undina*. Comparison of the deduced primary structure of the 16S rRNA of *A. distincta* revealed a distant relationship with *A. macleodii* (89.4 % sequence similarity), but closer relatedness with pseudoalteromonads, ranging from 94.5 % (*Pseudoalteromonas denitrificans*) to 99.7 % (*P. nigrifaciens*).

A. distincta possesses several features that are different from other previously described species of the genus *Pseudoalteromonas* and a table with differential characteristics has been published elsewhere (Ivanova *et al.*, 1998). Notably, the bacterium can be distinguished by the presence of both monotrichous cells and cells possessing two to five polar and lateral flagella. The species *P. denitrificans* has been found to have occasional cells with a tuft of two or three flagella (Enger *et al.*, 1987). On nutrient media, strain KMM 638^T produces an intracellular and extracellular diffusible, melanin-like pigment of the species belonging to the non-pigmented clade. It is worth noting that only *A. distincta* and *P. nigrifaciens* produce melanin (Ivanova *et al.*, 1996).

Although pseudoalteromonads utilize a wide range of compounds as a carbon source, strain KMM 638^T is unable to utilize glucose, sucrose, lactose, cellobiose, maltose, mannitol, *N*-acetylglucosamine, D-xylose, L-

Fig. 1. Unrooted neighbour-joining tree based on 1393 unambiguously aligned nucleotide positions. The numbers at the nodes exhibit the levels of bootstrap support based on neighbour-joining analyses of 1000 resampled data sets. The scale bar represents 0.01 nucleotide substitution per position. Nucleotide sequence accession numbers are X82145 (*A. macleodii*), AF043742 (*A. distincta*), X82142 (*Moritella marina*), X98336 (*P. antarctica*), X82134 (*P. atlantica*), X82135 (*Pseudoalteromonas aurantia*), D89929 (*Pseudoalteromonas bacteriolytica*), X82136 (*P. carrageenovora*), X82137 (*P. citrea*), X82138 (*P. denitrificans*), X82143 (*P. espejiana*), X67024 (*P. haloplanktis* subsp. *haloplanktis*), X82139 (*P. haloplanktis* subsp. *tetraodonis*), X82144 (*Pseudoalteromonas luteoviolacea*), X82146 (*P. nigrifaciens*), X82141 (*Pseudoalteromonas piscicida*), U85855 (*Pseudoalteromonas prydzensis*), X82147 (*Pseudoalteromonas rubra*), Z25522 (*Pseudoalteromonas tunicata*) and X82140 (*P. undina*).

arabinose, D-mannose or L-rhamnose. In addition, the number of complex substrates utilized by *A. distincta* is relatively small (Romanenko *et al.*, 1995; Ivanova *et al.*, 1998). DNA–DNA hybridizations carried out by Romanenko *et al.* (1995) and in this study showed the genetic uniqueness of *A. distincta*, when compared with other members of the genus *Pseudoalteromonas*. That is, DNA relatedness values of *A. distincta* and *P. undina*, *P. haloplanktis* subsp. *tetraodonis*, *P. haloplanktis* subsp. *haloplanktis*, *P. nigrifaciens*, *P. espejiana* and *Pseudoalteromonas citrea* were 16–41 %, and the corresponding value for *A. macleodii* was 10 % (Table 1).

Given the polyphasic data from the earlier study (Romanenko *et al.*, 1995) and presented here, it is proposed that *A. distincta* Romanenko *et al.* (1995) be assigned to the genus *Pseudoalteromonas* as *Pseudoalteromonas distincta* comb. nov.

Table 1. DNA relatedness among strains examined in this study

Species	G + C content (mol %)	Hybridization (%)			
		<i>P. distincta</i> ATCC 700518 ^T	<i>P. nigrifaciens</i> IAM 13010 ^T	<i>P. espejiana</i> IAM 12640 ^T	<i>P. haloplanktis</i> subsp. <i>haloplanktis</i> ATCC 14393 ^T
<i>P. distincta</i> ATCC 700518 ^T	43.8	100			
<i>P. nigrifaciens</i> IAM 13010 ^T	41.7	31	100		
<i>P. espejiana</i> IAM 12640 ^T	41.4	35	16	100	
<i>P. haloplanktis</i> subsp. <i>haloplanktis</i> ATCC 14393 ^T	42.7	30	33	21	100
<i>P. undina</i> IAM 12922 ^T	40.1	16	26	47	54
<i>P. haloplanktis</i> subsp. <i>tetraodonis</i> IAM 14160 ^T	41.5	17		18	48
<i>P. citrea</i> ATCC 29719 ^T	41.5	41	23	43	35
<i>P. atlantica</i> ATCC 19262 ^T	40.5		31	54	32
<i>P. antarctica</i> CECT 4664 ^T	42.1		49	31	19
<i>A. macleodii</i> ATCC 27126 ^T	45.3	10	9	12	12

Description of *Pseudoalteromonas distincta* (Romanenko, Mikhailov, Lysenko and Stepanenko) comb. nov.

The description of *Pseudoalteromonas distincta* comb. nov. is identical to that of Romanenko *et al.* (1995), with the addition that the species with four to seven lateral flagella be included. The description is based on a single strain, which is the type strain KMM 638^T (= ATCC 700518^T).

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