International Journal of Systematic and Evolutionary Microbiology (2000), **50**, 265–271 Printed in Great Britain

Assignment of *Alteromonas elyakovii* KMM 162^T and five strains isolated from spot-wounded fronds of *Laminaria japonica* to *Pseudoalteromonas elyakovii* comb. nov. and the extended description of the species

Tomoo Sawabe,[1] Reiji Tanaka,[1] Mohamed Mahabubu Iqbal,[1] Kenichi Tajima,[1] Yoshio Ezura,[1] Elena P. Ivanova[2] and Richard Christen[3]

Author for correspondence: Elena P. Ivanova. Fax: +7 4232 31 40 50.
e-mail: piboc@stl.ru *or* elenaivanova33@hotmail.com

[1] Laboratory of Microbiology, Faculty of Fisheries, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041, Japan

[2] Pacific Institute of Bioorganic Chemistry, Far Eastern Division, Russian Academy of Sciences, Vladivostok, 690022, Russia

[3] Centre National de la Recherche Scientifique et Université, Station Zoologique, Villefranche-sur-Mer 06230, France

A marine bacterium, *Alteromonas elyakovii* KMM 162^T, which was described recently, and five strains isolated from spot-wounded fronds of *Laminaria japonica* have been subjected to phylogenetic analysis, and geno- and phenotypic characterization. The phenotypic features of *Pseudoalteromonas elyakovii* strains were closely related to that of *Pseudoalteromonas espejiana* IAM 12640^T, but utilization of three carbon compounds (D-mannose, L-tyrosine and trehalose) distinguished both species. The G+C content of *Pseudoalteromonas elyakovii* was between 38·5 and 38·9 mol%. *Pseudoalteromonas elyakovii* KMM 162^T and the five *Laminaria* isolates constitute a single species different from any other *Alteromonas* and *Pseudoalteromonas* species as revealed by DNA–DNA hybridization data, especially *Pseudoalteromonas distincta* KMM 638^T (52·4%), *Pseudoalteromonas citrea* KMM 216 (49·5%), *Pseudoalteromonas carrageenovora* NCIMB 302^T (46·9%) and *Pseudoalteromonas espejiana* IAM 12640^T (29·9%). All the data indicated that *Alteromonas elyakovii* KMM 162^T should be reclassified as *Pseudoalteromonas elyakovii* and five strains isolated from *Laminaria japonica* have to be included in the species. *Pseudoalteromonas elyakovii* comb. nov. (type strain, KMM 162^T = ATCC 700519^T) is proposed and a set of phenotypic features which differentiate the *Pseudoalteromonas* species is described.

Keywords: *Alteromonas elyakovii*, *Pseudoalteromonas elyakovii* comb. nov., marine bacteria, spot-disease, *Laminaria*

INTRODUCTION

In the last 25 years, the taxonomy of *Alteromonas*-like bacteria has undergone a number of structural and nomenclatural amendments (Baumann *et al*., 1972, 1984; Van Landschoot & De Ley, 1983; Gauthier & Breittmayer, 1992; Gauthier *et al*., 1995). By phylogenetic analysis of 16S rDNA sequences, this group currently comprises the following genera: *Alteromonas*, *Pseudoalteromonas* (Gauthier *et al*., 1995) and *Glaciecola* (Bowman *et al*., 1998). The emended genus *Alteromonas* is presently restricted to two species, *Alteromonas macleodii* and *Alteromonas infernus* (Gauthier *et al*., 1995; Raguenes *et al*., 1995). The delineation of the species within the genus *Pseudoalteromonas* remains difficult because of their diverse pattern of breakdown of carbohydrates, the lack of useful chemotaxonomic markers and an insufficient degree of 16S rRNA gene sequence similarity. Therefore, a polyphasic approach based on DNA–DNA hybridization data is useful for identification of environmental isolates.

A marine bacterium, KMM 162, was isolated from the Far-Eastern mussel *Crenomytilus grayanus* collected in Troitsa Bay, Russia (Peter the Great Bay, The Sea of

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences reported in this paper are AF08562 and AF082562 for *Pseudoalteromonas elyakovii* KMM 162^T, AB000389 for IAM 14594 and AF116188 for O22. The number AF082563 is for *Pseudoalteromonas citrea* KMM 216 and AF082564 for *Pseudoalteromonas distincta* KMM 638^T.

Japan) in 1985 and the name *Alteromonas elyakovii* was proposed as a new species of *Alteromonas* (Ivanova *et al*., 1996). *Alteromonas elyakovii* KMM 162^T was interesting due to its ability to synthesize a highly active alkaline phosphatase and inducible laminarinase (Ivanova *et al*., 1996). At the same time of the isolation of the strain KMM 162, in 1985, an alginolytic marine bacterium was isolated from spot-wounded fronds of *Laminaria japonica* var. *ochotensis*, collected from Rishiri Island located at the eastern end of The Sea of Japan, 786 km from Troitsa Bay (Sawabe *et al*., 1992). The bacterium also assigned to the genus *Alteromonas* (Sawabe *et al*., 1992). The bacterium produced an extracellular alginate lyase showing broad substrate-specificity (Sawabe *et al*., 1997) and unique intracellular alginate-oligosaccharide-degrading enzymes (Sawabe *et al*., 1998c), and might be the causative agent of the spot disease. Later, in 1998, the spot disease of *Laminaria* occurred in the coastal area of Hokkaido, Japan, and several *Laminaria* frond-degrading bacteria have been isolated.

In a phylogenetic study of all species of the genera *Alteromonas*, *Shewanella* and '*Moritella*', leading to the creation of the genus *Pseudoalteromonas* (Gauthier *et al*., 1995), *Alteromonas elyakovii* was not included. In this study, DNA–DNA hybridizations, phenotypic characterizations and phylogenetic analyses were performed on *Alteromonas elyakovii* KMM 162^T and the marine bacteria isolated from spot-diseased fronds of *Laminaria*. All of the data suggest that these strains and *Alteromonas elyakovii* KMM 162^T belong in a single species that should be assigned to *Pseudoalteromonas elyakovii* as *P. elaykovii* comb. nov. From now on this new name will be used.

METHODS

Bacterial strains. Strains used in this study are listed in Table 1. *Pseudoalteromonas elyakovii* KMM 162^T (Ivanova *et al*., 1996), *Pseudoalteromonas citrea* KMM 216 (Romanenko *et al*., 1995; Ivanova *et al*., 1998) and *Pseudoalteromonas distincta* KMM 638^T (Romanenko *et al*., 1994; Ivanova *et al*., 2000) were provided by the Pacific Institute of Bioorganic Chemistry, Far Eastern Division, Russian Academy of Sciences (PIBOC FED RAS). *Pseudoalteromonas elyakovii* strains IAM 14594, a21, b11 and b211 (Sawabe *et al*., 1992) were isolated from wounded fronds of *Laminaria japonica* var. *ochotensis* in 1985, and strain O22 originated from *Laminaria japonica* fronds showing numerous spot lesions in 1998. All strains were cultured on ZoBell 2216E agar medium (Oppenheimer & ZoBell, 1952) and main characteristics were determined.

Morphological, biochemical and physiological characterization. Conventional phenotypic characteristics were determined by methods previously described (Baumann *et al*., 1984; Hidaka & Sakai, 1968; Holt *et al*., 1994; Leifson, 1963; Ostle & Holt, 1982; West *et al*., 1977). Alginate hydrolysis activity was determined by detecting the formation of a clear zone by flooding of a 70 % ethanol solution on the plate of ZoBell 2216E agar medium containing 0·5 % sodium alginate (Sawabe *et al*., 1995). Carrageenan hydrolysis activity was determined by a modified plate assay described by Yaphe & Baxter (1955). The colonies of bacteria which hydrolysed carrageenan were surrounded by an area of depression of the medium solidified with 3·0 % κ-carrageenan instead of agar.

Determination of mol % G+C content and DNA–DNA hybridizations. DNAs of bacterial strains were prepared by the procedures of Marmur (1961), with minor modification. DNA G+C contents were determined by the HPLC method (Tamaoka & Komagata, 1984). DNA–DNA hybridization experiments were performed in microdilution wells by the fluorometric direct binding method (Ezaki *et al*., 1988; Sawabe *et al*., 1998a, b). DNAs of *Pseudoalteromonas elyakovii* KMM 162^T and IAM 14594, *Pseudoalteromonas carrageenovora* NCIMB 302^T, *Pseudoalteromonas espejiana* IAM 12640^T and *Alteromonas macleodii* IAM 12920^T were labelled with photobiotin (Vector Laboratories). Unlabelled single-stranded DNAs were immobilized in microdilution wells (Immuron 200, FIA/LIA plate, black type; Greiner labotechnik), then hybridization mixture containing 20 ng labelled DNA was added to each microdilution well and the hybridization was performed under optimal condition following pre-hybridization and fluorometric detection using MicroFluoro reader (MTP-22; Corona Electric) previously described (Sawabe *et al*., 1998a, b). Formamide concentration in the hybridization mixture was determined according to Meinkoth & Wahl (1984) and DNA–DNA hybridization was calculated according to Ezaki *et al*. (1989).

DNA amplification and sequencing. The method used to prepare bacterial DNA for PCR was derived from the method of Enright *et al*. (1994). To amplify the small-subunit rRNA genes under the same conditions previously described (Sawabe *et al*., 1998a, b), 100 ng DNA template was used in a PCR. The PCR products, which gave a 1·5 kbp PCR product, were analysed on a 1·5 % agarose gel with a molecular mass standard, and PCR products which produced a single band on agarose gels were purified for sequencing by PEG 6000 precipitation. Approximately 100 ng template was directly sequenced by using a *Taq* FS dye terminator sequencing kit (ABI) and direct DNA sequencing was performed with an Applied Biosystems model 373A automated sequencer using nine primers as previously described by Sawabe *et al*. (1998a).

Phylogenetic analysis. Sequences were aligned and studied using a set of programs developed by one of us (R. Christen). In all phylogenetic analysis, we used the sequences determined in this study and small-subunit rDNA sequences obtained from the EMBL database. For Fig. 1, the following sequences were used: *Pseudoalteromonas antarctica* CECT 4664^T, X98336; *Pseudoalteromonas antarctica* N-1, AF045560; '*Pseudoalteromonas gracilis*' AF038846; *Pseudoalteromonas denitirificans* ATCC 43337^T, X82138; *Pseudoalteromonas citrea* NCIMB 1889^T, X82137; *Pseudoalteromonas aurantia* ATCC 33046^T, X82135; *Pseudoalteromonas atlantica* IAM 12927^T, X82134; *Pseudoalteromonas espejiana* NCIMB 2127^T, X82143; *Pseudoalteromonas carrageenovora* ATCC 12662^T, X82136; *Pseudoalteromonas undina* NCIMB 2128^T, X82140; *Pseudoalteromonas haloplanktis* ATCC 14393, X67024; *Pseudoalteromonas nigrifaciens* NCIMB 8614^T, X82146; *Pseudoalteromonas tetraodonis* IAM 14160^T, X82139; *Pseudoalteromonas piscicida* ATCC 15057^T, X82215; *Pseudoalteromonas piscicida* C201 CERBOM, X82141; *Pseudoalteromonas rubra* ATCC 29570^T, X82147; *Pseudoalteromonas luteoviolacea* NCIMB 1893^T, X82144; '*Pseudoalteromonas peptidysin*' F12-50-A1, AF007286; *Pseudoaltero-*

Table 1. Levels of DNA relatedness among *Pseudoalteromonas* and *Alteromonas* strains

Culture collections: IAM, IAM Culture Collection; NCIMB, National Collection of Industrial and Marine Bacteria; ATCC, American Type Culture Collection; KMM, Collection of Marine Microorganisms, Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences; CECT, Collection Espanola de Cultivos Tipo, Facultad de Ciencia Biologicas, Universidad de Valencia.

Organism	Strain	G+C content (mol%)	Reassociation (%) with biotinylated DNA from:				
			P. elyakovii KMM 162^T	*P. elyakovii* IAM 14594	*P. carrageenovora* NCIMB 302^T	*P. espejiana* IAM 12640^T	*A. macleodii* IAM 12920^T
P. elyakovii	KMM 162^T	38·5	100·0	78·1	38·3		
P. elyakovii	IAM 14594	38·8	94·3	100·0	45·8	43·1	7·6
P. elyakovii	a21	38·9	94·3	97·4	51·3		
P. elyakovii	b11	38·8	79·9	89·9	49·8		
P. elyakovii	b211	38·8	87·8	92·0	50·7		
P. elyakovii	O22	38·7	71·0	72·9	45·7		
P. citrea	KMM 216	43·8*	49·5	53·5	34·2		
P. distincta	KMM 638^T	43·8*	52·4	61·2	43·6		
A. macleodii	IAM 12920^T	45·3†		3·3	4·3		100·0
P. espejiana	IAM 12640^T	41·4†	29·9	35·3	41·2	100·0	4·0
P. atlantica	NCIMB 301^T	41·2†		36·8	34·7	54·5	4·8
P. carrageenovora	NCIMB 302^T	39·5†	46·9	45·8	100·0	46·4	3·8
P. antarctica	CECT 4664^T	40·6–41·7‡	13·5	36·1			
P. nigrifaciens	IAM 13010^T	40·6†		30·6	33·9	30·4	3·1
P. haloplanktis	IAM 12925^T	41·6†	28·9	28·8	42·7	29·1	3·8
P. haloplanktis	ATCC 19648	40·5†		31·7	36·0	31·9	5·4
P. undina	IAM 12922^T	40·1†		23·7	31·6	22·4	3·2
P. piscicida	NCMB 645^T	43–46§		9·2	13·8	10·6	5·3
P. rubra	ATCC 29570^T	46–48§		7·2	10·6	7·7	4·8
M. communis	IAM 12914^T	47·0†		4·0	8·5	2·6	1·9
M. vaga	IAM 12923^T	48·4†		3·1	9·2	2·9	1·3

* Data from Romanenko *et al*. (1995).

† Data from Romanenko *et al*. (1994).

‡ Data from Akagawa-Matsushita *et al*. (1992).

§ Data from Bozal *et al*. (1997).

monas prydzensis ACAM 620^T, U85855; *Pseudoalteromonas bacteriolytica* IAM 14595^T, D89929; *Alteromonas macleodii* subsp. *macleodii* IAM 12920^T, X82145; *Alteromonas macleodii* subsp. *fijiensis*, X85174; *Alteromonas infernus* CIP I-1628^T, X85175; *Moritella marina* NCIMB 1144^T, X82142; *Ferrimonas balearica* X93021; *Shewanella hanedai* CIP 103207^T, X82132; *Salinivibrio costicola* NCIMB 701^T, X95527; *Vibrio cholerae* CECT 514^T, X76337; *Escherichia coli* J01695; *Plesiomonas shigelloides* ATCC 7966^T, X60418; and *Aeromonas jandaei* X74678.

Domains used to construct the dendrogram shown in Fig. 1 were regions of the small-subunit rDNA sequences available for all sequences and excluding positions likely to show homoplasy: positions 101–181, 219–837, 850–1133, 1138–1205 (*E. coli* small-subunit rDNA sequence J01695 numbering). Phylogenetic analyses were performed by using three different methods, distances calculated using Kimura two-parameter corrections and a modified neighbour-joining method (BIONJ; Gascuel, 1997), maximum-likelihood (options QFYG, fdnaml program of G. J. Olsen, University of Illinois, Urbana, IL, USA) and maximum-parsimony (PHYLIP, Phylogeny Inference Package, version 3.5c. Distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle, WA, USA). The robustness of each topology was checked by using the neighbour-joining method and 100 bootstrap replications. Trees were drawn by using the NJPLOT program for the Macintosh (M. Gouy, CNRS URA 243, Université Claude Bernard, Lyon, France).

RESULTS AND DISCUSSION

Strains KMM 162^T, IAM 14594, a21, b11, b211 and O22 appeared as polarly flagellated, Gram-negative, strictly aerobic rods (Table 2). This bacterium required salt for its growth, did not accumulate poly-β-hydroxybutyrate (Table 2). No peritrichously flagellated cells were observed when the bacterium was cultivated on solid media. The growth temperature ranges from 10 to 37 °C, with optimum growth occurring at 25 °C. All of these characteristics, and the rather low G+C contents of the DNAs ranging from 38·5 to 38·9 mol% (Table 2), suggested that these strains belonged to the genera *Alteromonas* (Baumann *et al*., 1984) and *Pseudoalteromonas*.

Table 2. Phenotypic characteristics for distinguishing *Pseudoalteromonas elyakovii* from previously described alginolytic *Pseudoalteromonas* and *Alteromonas* species

All strains studied polarly flagellated bacteria, aerobic, require sodium ions for growth, are positive for oxidase and catalase, utilize D-glucose, do not utilize D-sorbitol or fucoidan. Strains: 1, *P. elyakovii* KMM 162^T; 2, *P. elyakovii* IAM 14594, a21, b11, b211, O22; 3, *P. espejiana* IAM 12640^T; 4, *P. citrea* KMM 216; 5, *P. carrageenovora* NCMB 302^T; 6, *P. atlantica* NCMB 301^T; 7, *P. distincta* KMM 638^T; 8, *P. haloplanktis* IAM 12915^T; 9, *A. macleodii* IAM 12920^T.

Character	Strain:								
	1	2	3	4	5	6	7	8	9
Pigmentation	−	−	−	+	−	+	+	−	−
Water-soluble						Brown	Brown		
Water-insoluble				Brown					
Growth at:	−	−	−	−	−	+		+	−
4 °C	−	d	+	−	+	−	−	+	−
37 °C	−	d	−	−	−	−	−	−	+
40 °C	−	−	−	−	−	−	−	−	+
Production of:									
Amylase	+	+	+	+	−	+	−	−	+
Alginase	+	+	+	+	+	+	+	−	+
Agarase	−	−	−	+	−	+	−	−	−
κ-Carrageenase	−	−	−	+	+	−	−	−	−
Utilization of:									
D-Mannose	+	+	−	+	−	+	+	−	−
D-Galactose	+	+	+	+	−	+	−	−	+
D-Fructose	+	+	+	+	+	+	+	−	+
Sucrose, maltose	+	+	+	+	+	−	+	−	+
Melibiose, lactose	+	+	+	+	+	+	−	−	+
D-Gluconate	−	+	−	−	−	−	−	+	+
N-Acetylglucosamine	−	d	−	−	−	−	−	−	+
Succinate, D-mannitol	+	+	+	+	+	−	+	+	+
Fumarate	+	+	+	+	+	−	+	+	−
Citrate	+	−	+	−	+	+	−	−	−
meso-Erythritol	−	−	−	−	−	−	−	+	−
Glycerol	−	+	+	−	+	+	+	+	−
γ-Aminobutyrate	−	−	−	−	−	−	−	−	+
L-Tyrosine	−	−	+	−	+	−	−	−	+
2-Oxoglutarate	−	−	−	−	−	−	−	+	−
Xylose	+	+	+	+	−	−	−	−	+
Trehalose	−	−	+	+	−	+	−	+	−
Acetate	+	+	+	+	+	+	−	+	+
D-Glucosamine	−	−	−	−	−	−	−	+	−
Pyruvate	+	+	+	+	+	+	+	−	+
Alginate	+	+	+	+	+	+	+	−	+
Laminarin	+	+	+	+	+	−	+	+	+
Aconitate, DL-malate, gluconate, δ-aminovarate	+	−	−	−	−	−	−	−	−

The results of a comparison of the phenotypic properties of these organisms with that of species of the genera *Alteromonas* and *Pseudoalteromonas* are shown in Table 2. The *Laminaria* isolates shared 67–93 % of phenotypic similarity with the following *Pseudoalteromonas* species, *Pseudoalteromonas carrageenovora* NCIMB 302^T, *Alteromonas macleodii* IAM 12920^T, *Pseudoalteromonas citrea* KMM 216, *Pseudoalteromonas distincta* KMM 638^T and *Pseudoalteromonas atlantica* NCIMB 301^T and *Pseudoalteromonas espejiana* IAM 12640^T(93 %) from which it differed only in three features: utilization of D-mannose, L-tyrosine and trehalose (Table 2). The similarities with other *Pseudoalteromonas* species were lower. The highest phenotypic similarity was found with *Alteromonas elaykovii* KMM 162^T; the *Laminaria* isolates and *Alteromonas elaykovii* 162^T were similar in their utilization of 16 organic compounds, amylase

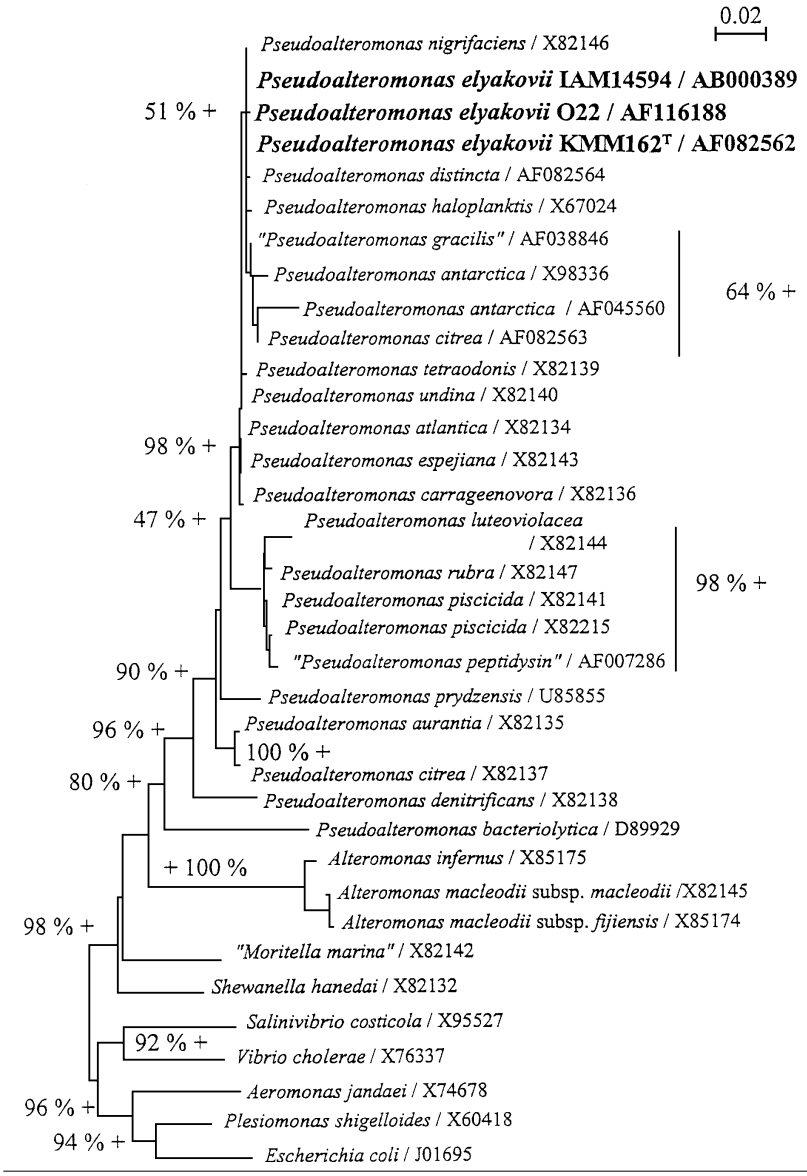

Fig. 1. Unrooted tree obtained using a neighbour-joining algorithm and a Kimura two-parameter correction for distance calculations. +, Branches also found in maximum-likelihood and parsimony analyses; %, bootstrap percentages retrieved in a 500 replications bootstrap analysis using Kimura two-parameter analysis.

and alginase production, and the G + C contents (98 % of phenotypic similarity).

DNA–DNA hybridization results showed that the level of DNA–DNA relatedness between *P. elaykovii* KMM 162^T and strains isolated from *Laminaria* fronds ranged from 71·0 to 94·3 %, between *P. elaykovii* IAM 14594, and the four *Laminaria* isolates (a21, b11, b211 and O22) the hybridization ranged from 72·9 to 97·4 % (Table 1). These data indicate that the strains belong to the same species (Wayne *et al.*, 1987). In contrast, none of these strains was related to any species of the genera *Pseudoalteromonas*, *Marinomonas* and *Alteromonas*. Distinctively low DNA–DNA homology values were found between strain *P. elaykovii* KMM 162^T and IAM 14594 and phenotypically closely related species, *Pseudoalteromonas espejiana* (29·9 %), *Pseudoalteromonas citrea* (49·5 %), *Pseudoalteromonas carrageenovora* (46·9 %) and *Pseudoalteromonas distincta* (52·4 %) (Table 1). DNA–DNA hybridization experiments effected with strain KMM 162^T, five *Laminaria* isolates and the four other species showed that strain KMM 162^T, and five strains that are the causative agent of spot disease of *Laminaria*, should be recognized as the same species.

In order to clarify the acute taxonomic affiliation of the *Alteromonas elyakovii* KMM 162^T and the *Laminaria* isolates, the 16S rDNA sequences of strains KMM 162^T, IAM 14594 and O22 were aligned by comparison to a database containing about 10000 already aligned eubacterial small-subunit rDNA sequences. The results of this phylogenetic analysis clearly showed that the strains which we studied belonged to the γ subclass of the class *Proteobacteria* of the domain *Bacteria* (data not shown), and more precisely, to the γ-3 subclass. More detailed analyses showed that these bacteria were included in the genus *Pseudoalteromonas*

and formed a cluster with *Pseudoalteromonas haloplanktis*, *Pseudoalteromonas nigrifaciens*, '*Pseudoalteromonas gracilis*', *Pseudoalteromonas citrea* KMM 216 and *Pseudoalteromonas antarctica*. The highest percentage of sequence similarity was found with *Pseudoalteromonas distincta* KMM 638^T (99·8–99·9 %) (Fig. 1).

The results allowed us unequivocally to assign the new isolates to the species *Alteromonas elyakovii* with a definite placement within the genus *Pseudoalteromonas* as a separate species. The name *Pseudoalteromonas elyakovii* comb. nov. is proposed for the strain KMM 162^T and five Japanese strains that are of high marine ecological importance in Far-Eastern sea area as they may induce severe damage to *Laminaria* crops.

Description of *Pseudoalteromonas elyakovii* comb. nov.

Pseudoalteromonas elyakovii (e.lya′ko.vi.i. M.L. gen. n. *elyakovii* after G. B. Elyakov, for his contribution to development of marine biotechnology in Russia).

Gram-negative, strictly aerobic, polarly flagellated bacterium isolated from the Far-Eastern mussel *Crenomytilus grayanus* and the wounded fronds of *Laminaria*. Rod-shaped cells with rounded ends, 0·5–0·8 μm in diameter and 1·8–4·0 μm long when the organism is grown on ZoBell 2216E agar medium; the cells occur singly or in pairs. Does not form endospores. Some strains are capsulated. Peritrichous flagellation is not observed when the organism is cultivated on solidified media. Colonies on ZoBell 2216E agar medium are beige, circular, and smooth and convex with entire edge. Sodium ion is essential for growth. Mesophilic and neutrophilic chemoorganotroph which grows at temperatures of 10–37 °C, with and optimum of 25–30 °C. No growth occurs at 40 °C. Positive for acid production from glucose; hydrolyses of starch, laminarin, gelatin, Tween 80 and alginate; oxidase; catalase; and assimilation of D-mannose, D-galactose, D-fructose, sucrose, maltose, melibiose, lactose, succinate, fumarate, D-mannitol, xylose, D-glucose, acetate, pyruvate, alginate and laminarin. Negative for denitrification; luminescence; production of fluorescein, pyocyanin and prodigiosin; hydrolyses of agar, chitin and κ-carrageenan; nitrate reduction; accumulation of poly-β-hydroxybutyrate; and assimilation of aconitate, meso-erythritol, γ-aminobutyrate, L-tyrosine, D-sorbitol, DL-malate, 2-oxoglutarate, trehalose, glucuronate, D-glucosamine, δ-aminovalerate and fucoidan. The DNA G+C content is 38·5–38·9 mol %. The type strain is KMM 162^T (=ATCC 700519^T).

ACKNOWLEDGEMENTS

We are grateful to Dr J. Guinea, Universitat de Barcelona who kindly provided the type strain *Pseudoalteromonas antarctica*. This work was partly supported a grant from Rishiru Town Museum.

REFERENCES

Akagawa-Matsushita, M., Matsuo, M., Koga, Y. & Yamasato, K. (1992). *Alteromonas atlantica* sp. nov. and *Alteromonas carrageenovora* sp. nov., bacteria that decompose algal polysaccharides. *Int J Syst Bacteriol* **42**, 621–627.

Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.

Baumann, P., Gauthier, M. J. & Baumann, L. (1984). Genus *Alteromonas* Baumann, Baumann, Mandel and Allen 1972, 418^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 343–352. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Bowman, J. P., McCammon, S. A., Brown, J. L. & McMeekin, T. A. (1998). *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea-ice habitats. *Int J Syst Bacteriol* **48**, 1213–1222.

Bozal, N., Tudela, E., Rosselló-Mora, R., Lalucat, J. & Guinea, J. (1997). *Pseudoalteromonas antarctica* sp. nov., isolated from an Antarctic coastal environment. *Int J Syst Bacteriol* **47**, 345–351.

Enright, M. C., Carter, P. E., MacLean, I. A. & McKenzie, H. (1994). Phylogenetic relationships between some members of the genera *Neisseria*, *Acinetobacter*, *Moraxella*, and *Kingella* based on partial 16S ribosomal DNA sequence analysis. *Int J Syst Bacteriol* **44**, 387–391.

Ezaki, T., Hashimoto, Y., Takeuchi, N., Yamamoto, H., Liu, S.-L., Miura, H., Matsui, K. & Yabuuchi, E. (1988). Simple genetic method to identify viridans group *Streptococci* by colorimetric dot hybridization and fluorometric hybridization in microdilution wells. *J Clin Microbiol* **26**, 1708–1713.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Gascuel, O. (1997). BIONJ, an improved version of the NJ algorithm based on a simple method of sequence data. *Mol Biol Evol* **14**, 685–695.

Gauthier, M. J. & Breittmayer, V. A. (1992). The genera *Alteromonas* and *Marinomonas*. In *The Prokaryotes, a Handbook on the Biology of Bacteria: Ecology*, *Isolation*, *Identification*, *Applications*, pp. 3046–3070. Edited by H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.

Gauthier, G., Gauthier, M. & Christen, R. (1995). 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. *Int J Syst Bacteriol* **45**, 755–761.

Hidaka, T. & Sakai, M. (1968). Comparative observation of inorganic salt requirement of the marine and terrestrial bacteria. *Bull Misaki Mar Biol Inst Kyoto Univ* **12**, 125–149.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (editors) (1994). Gram-negative aerobic/microaerophilic rods and cocci. In *Bergey's Manual of Determinative Bacteriology*, 9th edn, pp. 71–174. Baltimore: Williams & Wilkins.

Ivanova, E. P., Mikhailov, V. V., Kiprianova, E. A., Levanova, G. F., Garagulya, A. D., Frolova, G. M. & Svetashev, V. I. (1996). *Alteromonas elyakovii* sp. nov. a new bacterium isolated from marine mollusks. *Russ J Mar Biol* **22**, 209–215.

Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V. & 8 other authors (1998). Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description. *Int J Syst Bacteriol* **48**, 247–256.

Ivanova, E. P., Chun, J., Romanenko, L., Matte, M., Mikhailov, V., Frolova, G. M., Huq, A. & Colwell, R. R. (2000). Reclassification of *Alteromonas distincta* Romanenko *et. al.* 1995 as *Pseudoalteromonas distincta* comb. nov. *Int J Syst Evol Microbiol* **50**, 141–144.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **82**, 33–36.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Meinkoth, J. & Wahl, G. (1984). Hybridization of nucleic acids immobilized on solid supports. *Anal Biochem* **138**, 267–284.

Oppenheimer, C. H. & ZoBell, C. E. (1952). The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. *J Mar Res* **11**, 10–18.

Ostle, A. G. & Holt, J. G. (1982). Nile blue A as fluorescent stain for poly-β-hydroxybutyrate. *Appl Environ Microbiol* **44**, 238–241.

Raguenes, G., Pignet, P., Gauthier, G., Peres, A., Christen, R., Rougeaux, H., Barbier, G. & Guezennec, J. (1995). Description of a new polymer-secreting bacterium from a deep-sea hydrothermal vent, *Alteromonas macleodii* subsp. *fijensis*, and preliminary characterization of the polymer. *Appl Environ Microbiol* **62**, 67–73.

Romanenko, L. A., Lysenko, A. M., Mikhailov, V. V. & Kurika, A. V. (1994). A new species of brown-pigmented agarolytic bacteria of the genus *Alteromonas*. *Mikrobiologiya* **63**, 1081–1087 (in Russian).

Romanenko, L. A., Mikhailov, V. V., Lysenko, A. M. & Stepannenko, V. I. (1995). A new species of melanin-producing bacteria of the genus *Alteromonas*. *Mikrobiologiya* **64**, 74–77 (in Russian).

Sawabe, T., Ezura, Y. & Kimura, T. (1992). Characterization of an alginolytic marine bacterium from decaying Rishiri-kombu *Laminaria japonica* var. *ochotensis*. *Nippon Suisan Gakkaishi* **58**, 141–145.

Sawabe, T., Oda, Y., Shiomi, Y. & Ezura, Y. (1995). Alginate degradation by bacteria isolated from the gut of sea urchins and abalones. *Microb Ecol* **30**, 193–202.

Sawabe, T., Ohtsuka, M. & Ezura, Y. (1997). Novel alginate lyases from marine bacterium *Alteromonas* sp. H-4. *Carbohydr Res* **304**, 69–76.

Sawabe, T., Sugimura, I., Ohtsuka, M., Nakano, K., Tajima, K., Ezura, Y. & Christen, R. (1998a). *Vibrio halioticoli* sp. nov., a non-motile alginolytic marine bacterium isolated from the gut of the abalone *Haliotis discus hannai*. *Int J Syst Bacteriol* **48**, 573–580.

Sawabe, T., Makino, H., Tatsumi, M., Nakano, K., Tajima, K., Iqubal, M. M., Yumoto, I., Ezura, Y. & Christen, R. (1998b). *Pseudoalteromonas bacteriolytica* sp. nov., a marine bacterium that is the causative agent of red spot disease of *Laminaria japonica*. *Int J Syst Bacteriol* **48**, 769–774.

Sawabe, T., Sawada, C., Suzuki, E. & Ezura, Y. (1998c). Intracellular alginate-oligosaccharide degrading enzyme activity that is incapable of degrading intact sodium alginate from a marine bacterium *Alteromonas* sp. *Fish Sci* **64**, 320–324.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Van Landschoot, A. & De Ley, J. (1983). Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and some other Gram-negative bacteria. *J Gen Microbiol* **129**, 3057–3074.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37, 463–464.

West, M., Burdash, N. M. & Freimuth, F. (1977). Simplified silver plating stain for flagella. *J Clin Microbiol* **6**, 414–419.

Yaphe, W. & Baxter, B. (1955). The enzymatic hydrolysis of carrageenan. *Appl Microbiol* **3**, 380–383.