Pseudoalteromonas marina sp. nov., a marine bacterium isolated from tidal flats of the Yellow Sea, and reclassification of *Pseudoalteromonas sagamiensis* as *Algicola sagamiensis* comb. nov.

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Two Gram-negative, motile and strictly aerobic marine bacteria were isolated from a tidal flat sediment sample obtained from Dae-Chun, Chung-Nam, Korea. They were preliminarily identified as *Pseudoalteromonas*-like bacteria, based on 16S rRNA gene sequence analysis showing nearly identical sequences (>99.7 % sequence similarity) and the highest similarity (98.4 %) to the species *Pseudoalteromonas undina*. Some phenotypic features of the newly isolated strains were similar to those of members of the genus *Pseudoalteromonas*, but several physiological and chemo-taxonomical properties readily distinguished the new isolates from previously described species. DNA–DNA hybridization with type strains of phylogenetically closely related species demonstrated that the isolates represent a novel *Pseudoalteromonas* species, for which the name *Pseudoalteromonas marina* sp. nov. is proposed, with the type strain mano4^T (=KCTC 12242^T=DSM 17587^T). In addition, on the basis of this study and polyphasic data obtained from previous work, it is proposed that the species *Pseudoalteromonas sagamiensis* should be reclassified as *Algicola sagamiensis* comb. nov. and that strain B-10-31^T (=DSM 14643^T=JCM 11461^T) be designated the type strain.

The genus *Pseudoalteromonas* (type genus of the family *Pseudoalteromonadaceae*) (Gauthier *et al.*, 1995; Ivanova *et al.*, 2004a) currently comprises 34 recognized species, with *Pseudoalteromonas haloplanktis* as the type species. Many *Pseudoalteromonas* species have been isolated from the marine environment and show interstrain 16S rRNA gene sequence similarity values ranging from 90 to 99.9 %. To better describe the *Pseudoalteromonas* community associated with the tidal flats along the Korean coast (Kim *et al.*, 2004), marine sediment was sampled and its bacterial diversity was investigated. Here, we describe two novel *Pseudoalteromonas*-like strains, which were determined to belong to the genus *Pseudoalteromonas* on the basis of their 16S rRNA gene sequences. Accordingly, the objective of the present work was to elucidate the taxonomic position of these newly isolated strains, designated mano4^T and mano6, via phenotypic, genetic and chemo-taxonomic analyses. An additional aim was to determine whether the current taxonomic status of *Pseudoalteromonas sagamiensis* is appropriate.

Strains mano4^T and mano6 were isolated from a tidal flat area of Dae-Chun, Chung-Nam, Korea (36° 17′ 45.2″ N 126° 31′ 9.5″ E) using the dilution plating technique on marine agar 2216 (MA; Difco). The two strains were grown routinely at 25 °C for 3 days. Their closest relatives, as judged by 16S rRNA gene similarity, were *Pseudoalteromonas undina* DSM 6065^T, *Pseudoalteromonas translucida* DSM 14402^T and *Pseudoalteromonas aliena* DSM 16473^T. These three strains were obtained from DSMZ, Germany, and were grown under the same conditions and used as reference strains. Cultures of the isolates and the reference strains were stored at −80 °C in

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains mano4^T and mano6 are AY563031 and AY563032, respectively.

Table 1. Phenotypic characteristics that distinguish *P. marina* sp. nov. mano4^T from other species of the genus *Pseudoalteromonas*

Taxa: 1, *P. marina*; 2, *P. agarivorans*; 3, *P. aliena*; 4, *P. antarctica*; 5, *P. atlantica*; 6, *P. aurantia*; 7, *P. carrageenovora*; 8, *P. citrea*; 9, *P. denitrificans*; 10, *P. distincta*; 11, *P. elyakovii*; 12, *P. espejiana*; 13, *P. flavipulchra*; 14, *P. haloplanktis*; 15, *P. issachenkonii*; 16, *P. luteoviolacea*; 17, *P. maricaloris*; 18, *P. mariniglutinosa*; 19, *P. nigrifaciens*; 20, *P. paragorgicola*; 21, *P. peptidolytica*; 22, *P. phenolica*; 23, *P. piscicida*; 24, *P. prydzensis*; 25, *P. rubra*; 26, *P. ruthenica*; 27, *P. spongiae*; 28, *P. tetraodonis*; 29, *P. translucida*; 30, *P. tunicata*; 31, *P. ulvae*; 32, *P. undina*. Data were taken from this study and Ivanova *et al.* (2002c, 2004b), Romanenko *et al.* (2003a, b), Isnansetyo & Kamei (2003), Lau *et al.* (2005) and Egan *et al.* (2001). +, Positive; −, negative; +/−, variable reaction; W, weak reaction; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
DNA G+C content (mol%)	41.2	43.8	41.1	42.3	42.1	44.1	42	42.1	36.7	43.6	40	42.7	41.7	43.3	43	42	39.1	40.3	41.7	41.1	37.9	40.3	42.7	39	39	48.4	40.6	42.1	46.3	42	ND	42.2
Flagellation*	P	P	P	P	P	P	P	P	P	P, L	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	−	P	B	P	P	P
Pigmentation	−	−	+	−	−	+	−	+	+	+	−	−	+	−	−	+	+	−	+	+	+	+	+	−	+	+	−	−	−	+	+	−
Growth at/in:																																
4 °C	+	−	+	+	+	+	+	−	+	−	+	+/−	−	−	+	−	−	−	+	+	−	−	−	+	−	−	−	+	+	+	+	+
37 °C	+	−	−	−	−	−	+	−	−	−	+	+	+	−	+	+	+	+	−	−	+	+	+	−	+	+	+	−	−	−	−	−
1 % NaCl	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+
3−6 % NaCl	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
8 % NaCl	+	+	−	+	−	+	+	+	−	−	+	+	+	+	+	−	+	+	+	−	+	−	+	+	−	+	−	+	+	+	−	+
10 % NaCl	+	−	−	+	−	−	+	+	−	−	+	+	+	+	+	−	+	−	+	−	−	−	+	+	−	−	−	+	−	+	−	+
12 % NaCl	+	−	−	−	+/−	−	+	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+
15 % NaCl	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Production of:																																
Amylase	+	+	+	−	+	+	−	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	ND	+
Caseinase	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	ND	+	+	ND	+	+	+	ND	+
Alginase	+	+	+	ND	+	−	+	ND	+	+	+	+	ND	−	+	ND	+	+	+	ND	ND	−	ND	−	ND	+	−	ND	+	ND	−	−
Agarase	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Chitinase	−	−	ND	−	−	−	−	−	+	−	−	−	+	+/−	+	−	−	−	−	−	−	−	−	+	−	−	ND	−	−	−	ND	+
DNase	+	+	ND	+/−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	ND	+
Utilization of:																																
D-Glucose	+	W	W	+	+	+	+	+	+	+	+	+	+	+	+	+	+	W	+	ND	+	+	+	+	+	+	+	+	−	+	+	+
D-Arabinose	−	−	−	ND	ND	−	ND	−	−	ND	ND	ND	−	ND	ND	−	+	+	ND	+	−	−	−	ND	−	−	−	ND	+	−	−	ND
D-Galactose	−	−	−	−	+	−	−	−	ND	−	+	+	−	+/−	+	−	+	+	+	+	−	−	+	+/−	−	−	−	+	+	+	ND	−
Maltose	+	+	W	+	+	−	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	−	+	+	−	−	+	+
Melibiose	+	−	−	+	+	−	+	−	ND	+	−	+	−	−	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Lactose	−	−	ND	+	+	−	+	+	−	+	−	+	−	−	+	−	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−
D-Mannitol	−	−	W	+	+	−	+	−	−	−	+	+/−	−	+/−	+	−	+	+	+/−	+	−	−	−	+	−	−	−	−	+	−	ND	+/−
Sorbitol	−	ND	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	ND	−	−	−	−	−	−	−	−	−	−	ND	−	−	−
Citrate	−	−	−	+	+	−	+	−	ND	+	−	+	+	+	+	−	+	+	+	−	−	−	+	+	−	−	−	+	−	−	+	−
Xylose	−	−	−	ND	−	−	−	−	ND	−	+	+	−	−	−	−	−	−	+	ND	ND	−	−	−	−	−	−	−	−	−	−	−
L-Tyrosine	+	ND	−	ND	−	−	+	+	+	−	−	+	+	+	ND	ND	−	ND	+	−	ND	−	ND	+/−	−	−	ND	+	ND	ND	ND	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
L-Arginine	−	−	−	ND	ND	+	ND	−	ND	ND	ND	ND	−	ND	ND	+	+	−	ND	−	−	−	ND	ND	−	−	ND	ND	−	ND	ND	ND
Susceptibility to:																																
Ampicillin (10 μg)	−	+	−	−	+	+/−	−	−	ND	ND	+	−	−	+	−	−	−	−	+	+	+	ND	−	ND	−	−	+	+	−	+	+	+
Kanamycin (30 μg)	−	+	−	+	+/−	+	−	+/−	ND	ND	−	+	+	+	−	−	+/−	+	−	+	−	ND	+	ND	−	+/−	−	−	−	+	+	+
Carbenicillin (100 ED)†	+	−	−	+	−	+	+	−	ND	ND	+	+	+	+	+	+	ND	+	+	+	+	ND	+	ND	−	+	ND	+	−	+	+	+
Lincomycin (15 μg)	−	+	ND	−	+	+/−	−	−	ND	ND	−	−	−	−	−	−	−	−	+	−	−	ND	−	ND	−	−	ND	+	−	−	ND	−
Oleandomycin (15 μg)	+	+	+	−	+	+/−	+	+	ND	ND	+	+	−	+	+	+	+	ND	−	−	+/−	ND	+	ND	+	−	ND	−	+	+	ND	+
Polymyxin (300 ED)	+	ND	+	+	+	+	+	+	ND	ND	+	+	+	+	+	+	+	+	+	+	+	ND	+	ND	+	+	ND	+	+	+	ND	+
Streptomycin (10 μg)	+	ND	+	−	+	+/−	+	+/−	ND	ND	−	−	+/−	+	+	+	+	+	+	+	−	ND	+	ND	+	−	−	+	+	−	+	+
Tetracycline (30 μg)	−	−	ND	+	+	−	+	−	−	ND	+	+	−	+	+	−	−	−	+	−	−	ND	−	ND	−	−	+	+	−	−	+	+

*B, Bipolar; L, lateral; P, polar.
†ED, Effective dose.

marine broth (MB) containing 20 % glycerol. For morphological and physiological characterization, strains mano4^T and mano6 and the reference strains were generally cultivated in MB with shaking at 25 °C. API 20NE and API ZYM test strips (bioMérieux) were used to analyse the biochemical and physiological traits of these bacterial strains. Strips were inoculated with a heavy bacterial suspension in ASW or AUX medium (bioMérieux), supplemented with 2 % (w/v) sea salts. Other biochemical tests were performed using the methods and media described by Gordon *et al.* (1973). Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Growth under anaerobic conditions was determined microscopically (Nikon E600) after incubation for 7 days in anaerobic Gaspak jars (BBL) containing an atmosphere of 80 % N_2, 10 % CO_2 and 10 % H_2 (by vol.). Growth at various NaCl concentrations and at various temperatures and pH values was measured in MB. Cellular morphology and the presence of spores were also determined microscopically. Cellular motility for the novel isolate was examined using fresh wet-mounts of young bacterial cultures in MB. For TEM, cells from exponentially growing cultures were negatively stained with 1 % (w/v) phosphotungstic acid. After air-drying, the grid was examined using a model H-7600 transmission electron microscope (Hitachi). Results from the biochemical and physiological tests are given in Table 1 and in the species description. The new isolates could be readily differentiated from other closely related species by several phenotypic properties, as shown in Table 1.

Bacterial strains grown on MA for 3 days at 25 °C were used for the analysis of fatty acid methyl esters. The fatty acid methyl esters were extracted and prepared according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990). Chromosomal DNA was extracted and purified as described by Sambrook *et al.* (1989). DNA G+C content was assessed by using the methods described by Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed via HPLC using a reversed-phase column (Supelcosil LC-18-S; Supelco). Amplification, sequencing and phylogenetic analysis of the 16S rRNA gene were performed according to the methods described by Ivanova *et al.* (2002c). DNA–DNA hybridization was performed fluorometrically by using the method of Bae *et al.* (2005) with Cy5-labelled DNA probes and microarrays.

Phylogenetic trees based on 16S rRNA gene sequences of members of the genus *Pseudoalteromonas* showed that strains mano4^T and mano6 fall within the cluster of *Pseudoalteromonas* species (Fig. 1). Strains mano4^T and mano6 exhibited 16S rRNA gene sequence similarities of 92.8–98.4 % to the type strains of 34 other *Pseudoalteromonas* species.

DNA–DNA hybridization studies were performed to determine the genomic relationship between strains mano4^T and mano6 and their three closest relatives, *P.*

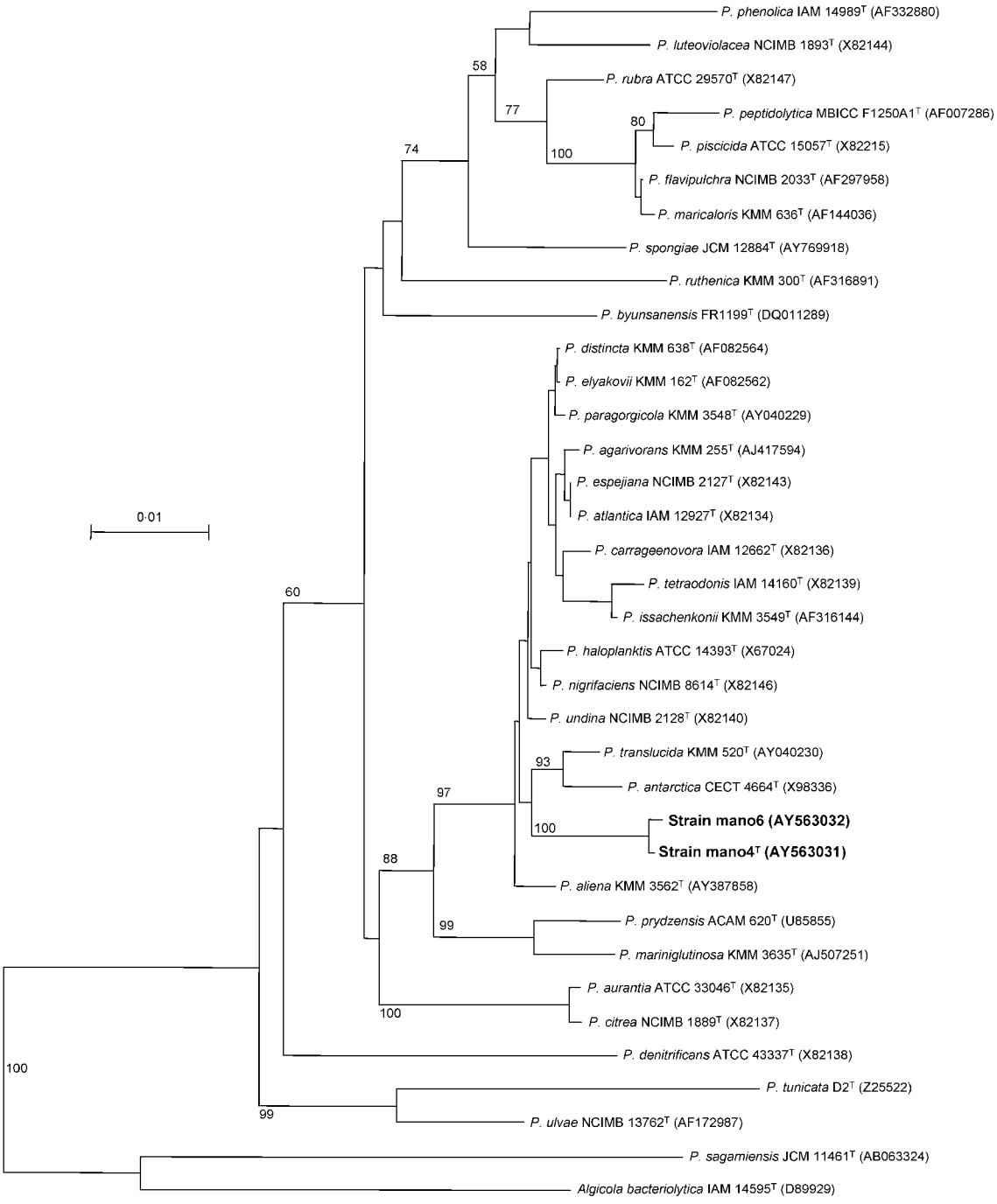

Fig. 1. Consensus phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strains mano4^T and mano6 and the type strains of other *Pseudoalteromonas* species. The tree was constructed based on the neighbour-joining method. Bootstrap analyses were performed with 1000 repetitions (only values greater than 50 % are shown). GenBank accession numbers are shown in parentheses. Bar, 1 substitution per 100 nucleotide positions.

undina DSM 6065^T, *P. translucida* DSM 14402^T and *P. aliena* DSM 16473^T, in terms of 16S rRNA gene similarity. We observed a mean level of 88 % DNA–DNA relatedness between strains mano4^T and mano6. The levels of DNA–DNA relatedness between strain mano4^T and its three closest relatives were all less than 4 %. Considering the phenotypic, phylogenetic and genotypic characteristics of the isolates, we concluded that strains mano4^T and mano6 belong within the genus *Pseudoalteromonas*, but are distinct from other recognized *Pseudoalteromonas* species described thus far. Considering also the phylogenetic and DNA–DNA hybridization data, we propose that the name of this novel species

should be *Pseudoalteromonas marina* sp. nov., and that strain mano4^T should be designated as the type strain.

Ivanova *et al.* (2000, 2002a, b, c, d, e, 2004b) have extensively explored the diversity and systematics of the genus *Pseudoalteromonas*. In successive studies, they observed that *Pseudoalteromonas bacteriolytica* Sawabe *et al.* 1998 (basonym of *Algicola bacteriolytica*) branched deeply in the phylogenetic tree of the genus and lacked a signature sequence (Ivanova *et al.*, 2004a). Thus, the genus *Algicola* has been newly proposed to resolve the phylogenetic relationships among the marine *Alteromonas*-like proteobacteria. However, *Pseudoalteromonas sagamiensis* (Kobayashi *et al.*, 2003), which is the species most closely related to *Algicola bacteriolytica*, was not included in the study. The 16S rRNA gene sequences of members of the genera *Pseudoalteromonas* and *Algicola* and a related species were retrieved from the NCBI database. DNA sequence alignment was conducted using CLUSTAL_X software (Thompson *et al.*, 1997). Phylogenetic trees were constructed using the Fitch & Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. The resultant unrooted tree topology was evaluated by bootstrap analysis based on 1000 resamplings (Felsenstein, 1985), using the neighbour-joining method. From the unrooted evolutionary tree shown in Fig. 1, we concluded that *P. sagamiensis* forms a branch with *Algicola bacteriolytica* that is distinct from other *Pseudoalteromonas* species. The relationship is based on data from different tree-making algorithms and the bootstrap value of 100 %. *P. sagamiensis* showed very low sequence similarity (90.4 %) to *Algicola bacteriolytica*. Despite this low similarity, Kobayashi *et al.* (2003) did not classify *P. sagamiensis* as representing a new genus because the phenotypic differences between *P. sagamiensis* and currently known related genera seemed too small to warrant generic separation.

P. sagamiensis can also be clearly differentiated by phenotypic characteristics determined in previous studies (Ivanova *et al.*, 2004a; Kobayashi *et al.*, 2003; Sawabe *et al.*, 1998). As shown in Table 2, *P. sagamiensis* and *Algicola bacteriolytica* showed low halotolerance, in contrast to the other *Pseudoalteromonas* species. They also shared the characteristic of lack of growth at 4 and 37 °C. However, some phenotypic properties, such as bacteriolytic activity and utilization of D-mannose, D-fructose and sucrose, differentiated *P. sagamiensis* from *Algicola bacteriolytica*.

Given the polyphasic data from an earlier study (Romanenko *et al.*, 1995) and the data presented here, we propose that *Pseudoalteromonas sagamiensis* Kobayashi *et al.* 2003 be reassigned to the genus *Algicola* as *Algicola sagamiensis* comb. nov.

Description of *Pseudoalteromonas marina* sp. nov.

Pseudoalteromonas marina (ma.ri′na. L. fem. adj. *marina* of the sea, marine).

Cells are Gram-negative, rod-shaped on MA (measuring 0.5–0.7 × 2.1–3.0 μm) and motile. Cells do not form endospores. Colonies are pale yellow in colour,

Table 2. Differential characteristics of members of the family *Pseudoalteromonadaceae*

+, Positive; −, negative; +/−, variable reaction; V, reaction varies between strains. Data from Ivanova *et al.* (2004a), Kobayashi *et al.* (2003) and Sawabe *et al.* (1998). All taxa are negative for flagella outer coat.

Characteristic	Genus *Pseudoalteromonas*	*Algicola bacteriolytica*	*Pseudoalteromonas sagamiensis*
Pigmentation	+/−	+	+
Flagellation:			
Bipolar	+/−	−	−
Lateral	+/−	−	−
Halotolerance (% NaCl)	15	6	5
Growth at:			
4 °C	+	−	−
37 °C	+	−	−
Hydrolysis of:			
Chitin	V	−	−
Starch	V	+	+
Utilization of:			
D-Fructose	V	+	−
D-Mannose	V	+	−
Sucrose	V	+	−
Glycerol	V	−	−
Lactose	V	−	−
DNA G+C content (mol%)	37–50	44–46	42

0.2–0.5 mm in diameter, smooth and circular to slightly irregular in shape after 3 days of culture on MA. Growth occurs at 4–37 °C and at pH 5.3–8.8, but not at pH values lower than 4.1 nor higher than 9.3. Growth occurs in the presence of 3–12 % NaCl, but not in the absence of NaCl or in 15 % NaCl. Growth is not observed under anaerobic conditions. Catalase-positive and Voges–Proskauer test negative. Casein and starch are hydrolysed, but nitrate is not reduced to nitrite. Produce amylase, caseinase, alginase, DNase, but not agarase or chitinase, and utilize glucose, maltose and melibiose as a sole carbon and energy source. The following substrates are not utilized: D-arabinose, L-arginine, D-galactose, lactose, D-mannitol, sorbitol, citrate and xylose. Major fatty acids are $C_{15:0}$ (6.8 ± 0.4 %), $C_{16:0}$ (21.3 ± 0.7 %) and $C_{16:1}\omega7c$ (24.7 ± 1.2 %).

The DNA G+C content of the type strain is 41.2 mol%. The type strain, mano4^T (=KCTC 12242^T=DSM 17587^T), was isolated from a tidal flat area of Dae-Chun, Chung-Nam, Korea.

Description of *Algicola sagamiensis* Kobayashi *et al.* 2003 comb. nov.

Algicola sagamiensis (sa.ga.mi.en′sis. N.L. fem. adj. *sagamiensis* referring to Sagami Bay, the place of isolation).

Basonym: *Pseudoalteromonas sagamiensis* Kobayashi *et al.* 2003. The description is identical to that given by Kobayashi *et al.* (2003).

The type strain is B-10-31^T (=DSM 14643^T=JCM 11461^T).

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