Pseudoalteromonas spongiae sp. nov., a novel member of the γ -Proteobacteria isolated from the sponge *Mycale adhaerens* in Hong Kong waters

Stanley C. K. Lau,¹ Mandy M. Y. Tsoi,¹ Xiancui Li,¹ Sergey Dobretsov,¹ Yulia Plakhotnikova,¹ Po-Keung Wong² and Pei-Yuan Qian¹

Correspondence

Pei-Yuan Qian

boqianpy@ust.hk

¹Coastal Marine Laboratory/Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

²Department of Biology, Chinese University of Hong Kong, Shatin, N.T., Hong Kong

A Gram-negative, non-spore-forming, short rod-shaped bacterium (UST010723-006^T) was isolated from the surface of the sponge *Mycale adhaerens* in Hong Kong waters. Cells of UST010723-006^T did not have flagella and were non-motile. Colonies were pale orange in colour, 2–4 mm in diameter, convex with a smooth surface and an entire translucent margin. Gas bubbles were observed in the colonies and also in the agar matrix underneath and adjacent to the colonies. UST010723-006^T was heterotrophic, strictly aerobic and required NaCl for growth (2.0–6.0%). It grew at pH 5.0–10.0 and between 12 and 44 °C. Phylogenetic analysis of the 16S rRNA gene sequence placed UST010723-006^T within the genus *Pseudoalteromonas* of the γ -subclass of the Proteobacteria. The DNA G+C content is 40.6 mol% and the dominant fatty acids were 12:0 3-OH, 14:0, 15:0 iso 2-OH, 16:0, 16:1 ω 7, 17:1 ω 8 and 18:1 ω 7 (altogether representing 75.9% of the total). These data supported the affiliation of UST010723-006^T to the genus *Pseudoalteromonas*. The closest relatives were *Pseudoalteromonas luteoviolacea*, *P. phenolica*, *P. rubra* and *P. rutenica* with similarity values ranging from 95.4 to 96.8%. UST010723-006^T differed from these closest relatives by 9–19 traits. Molecular evidence, together with phenotypic characteristics, suggests that UST010723-006^T constitutes a novel species within the genus *Pseudoalteromonas*. The name *Pseudoalteromonas spongiae* sp. nov. is proposed for this bacterium. The type strain is UST010723-006^T (=NRRL B-41100^T=JCM 12884^T).

The genus *Pseudoalteromonas* currently comprises 32 described members; many isolated from marine macroorganisms, such as *Pseudoalteromonas rutenica* from mussels and scallops (Ivanova *et al.*, 2002a), *Pseudoalteromonas maricalaris* from sponges (Ivanova *et al.*, 2002b), *Pseudoalteromonas tunicata* from tunicates (Holmström *et al.*, 1998) and *Pseudoalteromonas ulvae* from algae (Egan *et al.*, 2001). They are often suggested to have important ecological implications for the survival and fitness of the host organism (Holmström *et al.*, 2002). In this study, we describe a novel member of *Pseudoalteromonas* isolated from the surface of a sponge in subtropical water.

During the characterization of bacteria isolated from the surface of the sponge *Mycale adhaerens* in Hong Kong waters, the strain UST010723-006^T was isolated on nutrient agar (Oxoid) supplemented with 0.22- μ m-filtered sea water (referred to as marine agar) at 30 °C. Unless otherwise specified, all characteristics described hereafter were based on cultures grown on marine agar for 48 h at 30 °C. UST010723-006^T appeared as pale orange, convex colonies with an entire translucent margin and a smooth surface. No diffusible pigment was observed. Gas bubbles were observed in the colonies as well as in the agar matrix underneath and adjacent to the colonies; this property has not been reported for any of the already described members of *Pseudoalteromonas*.

Published online ahead of print on 4 March 2005 as DOI 10.1099/ijs.0.63638-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST010723-006^T is AY769918.

Supplementary material available in IJSEM Online comprises a table showing primers used for the construction of 16S rRNA gene sequences, TEM and SEM images and a table detailing the carbohydrate metabolism of strain UST010723-006^T.

The nearly complete 16S rRNA gene sequence of UST010723-006^T (1425 bp) was resolved on a MegaBACE capillary genetic analyser using a dye terminator method according to the manufacturer's protocol. Primers used in the sequencing reactions are detailed in Supplementary Table S1 available in IJSEM Online. Fragments of DNA

Fig. 1. Neighbour-joining dendrogram showing the estimated phylogenetic relationships among UST010723-006^T and related *Pseudoalteromonas* species on the basis of 16S rRNA gene sequences. *Vibrio marinus* ATCC 15381^T was chosen as the outgroup. Scale bar represents 1 nucleotide substitution per 100 nucleotides. Bootstrap values of >50% (500 replicates) are indicated at nodes. The GenBank accession number for each reference strain is shown in parentheses.

sequence obtained from individual primers with at least six replicates were assembled using the Sequencher software package (Gene Codes). Comparison of the 16S rRNA gene sequence of UST010723-006^T with those available from GenBank revealed that UST010723-006^T was a member of the γ -subclass of the *Proteobacteria* and shared 99.2% sequence similarity with *Pseudoalteromonas* sp. S9 (Techkarnjanaruk *et al.*, 1997). A neighbour-joining phylogenetic tree (Fig. 1) constructed using the ARB software package (Ludwig *et al.*, 2004) showed that UST010723-006^T and *Pseudoalteromonas* sp. S9 belonged to a clade (100% bootstrap value, 500 replications), that did not cluster robustly with any recognized species of *Pseudoalteromonas* (63% bootstrap value, 500 replications). Trees based on maximum-parsimony and maximum-likelihood methods showed essentially the same topology (data not shown). The results of phylogenetic analysis suggest that UST010723-006^T represents a novel species within the genus *Pseudoalteromonas*. The closest relatives of UST010723-006^T were *Pseudoalteromonas luteoviolacea* (Gauthier, 1982), *P. rutenica* (Ivanova *et al.*, 2002a), *P. rubra* (Gauthier, 1976) and *P. phenolica* (Isnansetyo & Kamei, 2003) with 96.8, 96.6, 96.5 and 95.4% sequence identity, respectively.

The dominant cellular fatty acids of UST010723-006^T are 12:0 3-OH, 14:0, 15:0 iso 2-OH, 16:0, 16:1 ω 7, 17:1 ω 8 and 18:1 ω 7 (altogether representing 75.9% of the total; three replications) as determined using the Sherlock Microbial Identification System. This fatty acid profile is similar to that of *P. rutenica* (Table 1). No fatty acid profiles have been reported for *P. phenolica*, *P. luteoviolacea* or *P. rubra*. The DNA G + C content of the strains UST010723-006^T as determined by the HPLC method of Mesbah *et al.* (1989) was 40.6 $\pm$ 0.2 mol%. This value was similar to those described for *P. luteoviolacea* and *P. phenolica* (Table 2).

The phenotypic characteristics of UST010723-006^T are given in the species description. Anaerobic growth was examined in the Oxoid Anaerobic System. The requirement for NaCl was tested in a medium containing (per litre) 5 g MgCl₂, 2 g MgSO₄, 0.5 g CaCl₂, 1 g KCl, 5 g peptone and various amounts of NaCl adjusted to pH 7.5 using KOH (Isnansetyo & Kamei, 2003). Cell morphology was examined using scanning electron microscopy (JEOL 7600F) according to Neu *et al.* (2001). The presence of flagella was

examined using light microscopy after Leifson staining according to Smibert & Krieg (1994) and using transmission electron microscopy (TEM) (JEOL 100 CX) (Grossart *et al.*, 2000) (scanning electron microscopy and TEM pictures are available as Supplementary Fig. S1 in IJSEM Online). Reaction to Gram-stain was determined using light microscopy according to Smibert & Krieg (1994). Cell motility was determined using both light microscopy and observation of growth in motility medium (Smibert & Krieg, 1994). Susceptibility to antibiotics was tested according to Acar (1980). Agarase, amylase, catalase, DNase and oxidase activities were tested using the method of Smibert & Krieg (1994). Substrate utilization patterns and other enzyme activities were tested with the commercial systems API 20E, API 20NE, API 50CH and API ZYM (bioMérieux). Cells for inoculation to the API systems were suspended in sterile solution of sea water mixture at 22‰ salinity (MacDonell *et al.*, 1982).

UST010723-006^T differs from all previously described members of *Pseudoalteromonas* by testing negative for lipase activity and by the formation of gas bubbles in the

Table 1. Dominant cellular fatty acids of UST010723-006^T and *Pseudoalteromonas rutenica*

Numbers given are the mean percentages ($\pm$ SD) of total fatty acids. Data for *Pseudoalteromonas rutenica* are from Ivanova *et al.* (2002a). NR, not reported by Ivanova *et al.* (2002a).

Dominant fatty acid	Content (%)	
	UST010723-006 ^T	<i>P. rutenica</i>
12:0 3-OH	6.9 $\pm$ 1.2	NR
14:0	5.3 $\pm$ 1.8	2.0–11.0
15 iso 2-OH	29.1 $\pm$ 5.9*	NR
16:1 ω 7	29.1 $\pm$ 5.9*	31.0–42.0
16:0	18.4 $\pm$ 2.9	13.8–18.7
17:1 ω 8	9.8 $\pm$ 5.3	4.0–8.0
18:1 ω 7	6.4 $\pm$ 1.2	4.0–16.0

*Appeared as a summed feature comprised of 15 iso 2-OH and 16:1 ω 7.

colonies (Table 1) (Gauthier *et al.*, 1995). UST010723-006^T can be distinguished from the four closest relatives (*P. luteoviolacea*, *P. ruthenica*, *P. rubra* and *P. phenolica*) by 9–19 traits, particularly the lack of amylase activity, flagella and cellular motility (Table 2). Molecular evidence, together with phenotypic characteristics, suggests that UST010723-006^T constitutes a novel species within the genus *Pseudoalteromonas*.

Description of *Pseudoalteromonas spongiae* sp. nov.

Pseudoalteromonas spongiae (spon'gi.ae. L. gen. n. *spongiae* of a sponge).

Cells are Gram-negative, short rods (1.2–1.5 µm in length and 0.5 µm in width) and do not form spores. Cells do not have flagella and are non-motile. Upon cultivation on

Table 2. Differentiation of UST010723-006^T from other recognized members of *Pseudoalteromonas*

Data for *P. ruthenica*, *P. luteoviolacea*, *P. rubra* and *P. phenolica* are from Ivanova *et al.* (2002a), Gauthier (1982), Gauthier (1976) and Isnansetyo & Kamei (2003), respectively. +, Positive; (+), weakly positive; –, negative; v, variable; ND, not described. All are oxidase-, DNase- and gelatinase-positive, but are arginine dihydrolase-negative; all utilize glucose, but not D-adonitol, D-arabinose, erythritol, D-galactose, glycerol, D-lactose, D-mannitol, L-rhamnose or D-sorbitol.

Characteristic	UST010723-006 ^T	<i>P. ruthenica</i>	<i>P. luteoviolacea</i>	<i>P. rubra</i>	<i>P. phenolica</i>
Colony					
Colour	Pale orange	Pale orange	Purple/yellow	Red	Brown
Diffusible pigment	–	–	–	Orange–brown	ND
Gas bubble formation	+	ND	ND	ND	ND
Cell					
Flagella	–	+	+	+	+
Motility	–	+	+	+	+
Growth:					
NaCl (%)	2.0–6.0	1.0–9.0	3.0–6.0	2.0–6.0	1.0–5.0
Temperature (°C)	8.0–44.0	10.0–35.0	10.0–30.0	10.0–37.0	18.0–37.0
pH	5.0–10.0	6.0–10.0	>6.0	6.0–10.0	6.5–9.5
G + C content (mol%)	40.6	48.4–48.9	40.9–42.2	46.7–47.3	39.9–40.6
Production of:					
Amylase	–	+	+	+	+
Catalase	+	+	–	(+)	–
Esterase	+ ^{a*}	ND	ND	–	ND
Lipase	– ^a	+	ND	+	+
Lysine decarboxylase	– ^b	ND	ND	v	ND
Tryptophan deaminase	– ^b	ND	ND	+	ND
Utilization of:					
N-Acetylglucosamine	+ ^c	–	ND	ND	+
D-Cellobiose	– ^c	+	–	–	ND
D-Fructose	+ ^c	–	–	–	ND
Inositol	+ ^c	–	–	–	–
Inulin	+ ^c	–	–	–	ND
Maltose	+ ^c	–	+	–	+
Mannose	+ ^c	–	–	+	+
Sucrose	– ^c	+	–	–	+
D-Trehalose	– ^c	–	+	+	+
Susceptible to:					
Kanamycin (30 µg)	– (100.0 µg)	v	–	(+)	ND
Streptomycin (10 µg)	– (100.0 µg)	–	–	(+)	ND
Benzylpenicillin†	+	–	–	–	ND
Tetracycline†	+	ND	–	(+)	ND
Ampicillin†	+	–	ND	ND	ND

*Result based on: a, API ZYM; b, API 20E; c, API 50CH.

†Concentration not reported unless specified.

marine agar, colonies are pale orange in colour, 2–4 mm in diameter, convex with a smooth surface and an entire translucent margin. Gas bubbles are observed in the colonies and also in the agar matrix underneath and adjacent to the colonies. Growth of UST010723-006^T is strictly aerobic and occurs between 12 and 44 °C (but not at 4 or 52 °C) and between pH 5.0 and 10.0. UST010723-006^T requires NaCl (2.0–6.0%) for growth. The DNA G+C content is 40.6 mol% and the dominant fatty acids are 12:0 3-OH, 14:0, 15:0 iso 2-OH, 16:0, 16:1 ω 7, 17:1 ω 8 and 18:1 ω 7 (altogether representing 75.9% of the total). Susceptible to benzylpenicillin (0.1 µg), chloramphenicol (0.1 µg), ampicillin (0.1 µg) and tetracycline (1 µg). Resistant to streptomycin and kanamycin (tested up to 100 µg). Acetoin, indole and H₂S are not produced. Citrate is not utilized. Nitrate is not reduced. α -Chymotrypsin, acid phosphatase, alkaline phosphatase, catalase, DNase, esterase (C4), esterase lipase (C8), gelatinase, leucine arylamidase, oxidase, protease and trypsin activities are positive. *N*-Acetyl- β -glucosaminidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase activities are weakly positive. α -Fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, α -mannosidase, β -glucuronidase, agarase, amylase, arginine dihydrolase, cystine arylamidase, lipase (C14), lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease activities are negative. Grows on D-glucose, D-fructose, inositol, D-mannose, D-maltose, inulin, *N*-acetylglucosamine and starch as sole carbon source. Acid is produced from D-maltose and *N*-acetylglucosamine. Carbohydrates not utilized by UST010723-006^T are listed in Supplementary Table S2 available in IJSEM Online.

The type strain, UST010723-006^T (=NRRL B-41100^T=JCM 12884^T), was isolated from the surface of the sponge *Mycale adhaerens* in Hong Kong waters.

Acknowledgements

This work was supported by a Central Allocation Grant of Hong Kong Research Grant Council (CA04/05. Sc01) to P.-Y. Q.

References

- Acar, J. F. (1980). The disc susceptibility test. In *Antibiotics in Laboratory and Medicine*, pp. 24–54. Edited by V. Lorian. Baltimore: Williams & Wilkins.
- Egan, S., Holmström, C. & Kjelleberg, S. (2001). *Pseudoalteromonas ulvae* sp. nov., a bacterium with antifouling activities isolated from surface of a marine alga. *Int J Syst Evol Microbiol* **51**, 1499–1504.
- Gauthier, M. J. (1976). *Alteromonas rubra* sp. nov., a new marine antibiotic-producing bacterium. *Int J Syst Bacteriol* **26**, 459–466.
- Gauthier, M. J. (1982). Validation of the name *Alteromonas luteoviolacea*. *Int J Syst Bacteriol* **32**, 82–86.
- Gauthier, G., Gauthier, M. & Christen, R. (1995). Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes 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.
- Grossart, H. P., Steward, G. F., Martinez, J. & Azam, F. (2000). A simple, rapid method for demonstrating bacterial flagella. *Appl Environ Microbiol* **66**, 3632–3636.
- Holmström, C., James, S., Neilan, B. A., White, D. C. & Kjelleberg, S. (1998). *Pseudoalteromonas tunicata* sp. nov., a bacterium that produces antifouling agents. *Int J Syst Bacteriol* **48**, 1205–1212.
- Holmström, C., Egan, S., Franks, S., McCloy, S. & Kjelleberg, S. (2002). Antifouling activities expressed by marine surface associates *Pseudoalteromonas* species. *FEMS Microbiol Ecol* **41**, 47–58.
- Isnansetyo, A. & Kamei, Y. (2003). *Pseudoalteromonas phenolica* sp. nov., a novel marine bacterium that produces phenolic antimethicillin-resistant *Staphylococcus aureus* substances. *Int J Syst Evol Microbiol* **53**, 583–588.
- Ivanova, E. P., Sawabe, T., Lysenko, A. M. & 8 other authors (2002a). *Pseudoalteromonas ruthenica* sp. nov., isolated from marine invertebrates. *Int J Syst Evol Microbiol* **52**, 235–240.
- Ivanova, E. P., Shevchenko, L. S., Sawabe, T., Lysenko, A. M., Svetashev, V. I., Gorshkova, N. M., Satomi, M., Christen, R. & Mikhailov, V. V. (2002b). *Pseudoalteromonas maricaloris* sp. nov., isolated from an Australian sponge, and reclassification of [*Pseudoalteromonas aurantia*] NCIMB 2033 as *Pseudoalteromonas flavipulchra* sp. nov. *Int J Syst Evol Microbiol* **52**, 263–271.
- Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- MacDonell, M. T., Singleton, F. L. & Hood, M. A. (1982). Diluent composition for use of API 20E in characterizing marine and estuarine bacteria. *Appl Environ Microbiol* **44**, 423–427.
- Mesbah, M., Premachandran, U. & Whitman, W. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Neu, B., Voigt, A., Mitlohner, R. & 7 other authors (2001). Biological cells as templates for hollow microcapsules. *J Microencapsul* **18**, 385–395.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characteristics. In *Methods for General and Molecular Biology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Techkarnjanaruk, S., Pongpattanakitsote, S. & Goodman, A. E. (1997). Use of a promoterless lacZ gene insertion to investigate chitinase gene expression in the marine bacterium *Pseudoalteromonas* sp. strain S9. *Appl Environ Microbiol* **63**, 2989–2996.