Alteromonas tagae sp. nov. and *Alteromonas simiduii* sp. nov., mercury-resistant bacteria isolated from a Taiwanese estuary

Hsiu-Hui Chiu,[1] Wung Yang Shieh,[1] Silk Yu Lin,[1] Chun-Mao Tseng,[1,2] Pei-Wen Chiang[2] and Irene Wagner-Döbler[3]

Correspondence
Wung Yang Shieh
winyang@ntu.edu.tw

[1]Institute of Oceanography, National Taiwan University, PO Box 23-13, Taipei, Taiwan

[2]National Center for Ocean Research, National Taiwan University, PO Box 23-13, Taipei, Taiwan

[3]GBF – Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig, Germany

Two mercury-resistant strains of heterotrophic, aerobic, marine bacteria, designated AT1^T and AS1^T, were isolated from water samples collected from the Er-Jen River estuary, Tainan, Taiwan. Cells were Gram-negative rods that were motile by means of a single polar flagellum. Buds and prosthecae were produced. The two isolates required NaCl for growth and grew optimally at about 30 °C, 2–4 % NaCl and pH 7–8. They grew aerobically and were incapable of anaerobic growth by fermenting glucose or other carbohydrates. They grew and expressed Hg^{2+}-reducing activity in liquid media containing $HgCl_2$. Strain AS1^T reduced nitrate to nitrite. The predominant isoprenoid quinone was Q_8 (91.3–99.9 %). The polar lipids of strain AT1^T consisted of phosphatidylethanolamine (46.6 %), phosphatidylglycerol (28.9 %) and sulfolipid (24.5 %), whereas those of AS1^T comprised phosphatidylethanolamine (48.2 %) and phosphatidylglycerol (51.8 %). The two isolates contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (22.4–33.7 %), $C_{16:0}$ (19.0–22.7 %) and $C_{18:1}\omega7c$ (11.3–11.7 %) as the major fatty acids. Strains AT1^T and AS1^T had DNA G+C contents of 43.1 and 45.3 mol%, respectively. Phylogeny based on 16S rRNA gene sequences, together with data from morphological, physiological and chemotaxonomic characterization, indicated that the two isolates could be classified as representatives of two novel species in the genus *Alteromonas*, for which the names *Alteromonas tagae* sp. nov. (type strain AT1^T=BCRC 17571^T=JCM 13895^T) and *Alteromonas simiduii* sp. nov. (type strain AS1^T=BCRC 17572^T=JCM 13896^T) are proposed.

Mercury is one of the most toxic elements to all living organisms. This toxicity is due to its binding to the thiol groups of enzymes and other proteins, thereby inactivating vital cell functions (Wagner-Döbler *et al.*, 2000). The major form of mercury in the atmosphere is elemental mercury (Hg^0), which is volatile and is oxidized to the mercuric ion (Hg^{2+}) as a result of its interaction with ozone in the presence of water (Munthe, 1992). Thus, most of the mercury entering the aquatic environment is Hg^{2+}. Inorganic mercury species, Hg^{2+} and Hg^0, present in the aquatic environment are subjected to microbiological conversion to highly toxic methyl mercury compounds that are subsequently bioaccumulated through the food chain. The health of predatory organisms at the top of the food chain, such as fish, birds and humans, is thereby threatened (Muir *et al.*, 1999).

Mercury-resistant bacteria are usually aerobes or facultative anaerobes, which are readily isolated on a variety of media from water, soil and sediment as well as from humans and other animals (Osborn *et al.*, 1997; Barkay *et al.*, 2003). They play a major role in the global cycling of mercury. The mechanism of their mercury resistance is mediated by cytoplasmic mercuric reductase, which converts soluble Hg^{2+} to insoluble Hg^0, followed by volatilization of the relatively non-toxic Hg^0; the mercuric reductase is encoded by the *merA* gene (Silver & Phung, 1996). Various genera such as *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Bacteriodes*, *Beijerinckia*, *Caulobacter*, *Chromobacterium*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Erwinia*, *Escherichia*, *Exiguobacterium*, *Flavobacterium*, *Klebsiella*, *Moraxella*, *Morganella*, *Mycobacterium*, *Paracoccus*, *Proteus*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodococcus*,

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Alteromonas tagae* AT1^T and *Alteromonas simiduii* AS1^T are DQ836765 and DQ836766, respectively.

Salmonella, *Serratia*, *Shewanella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Streptomyces*, *Thiobacillus*, *Xanthomonas* and *Yersinia* are reported to include mercury-resistant bacteria (Ji *et al.*, 1989; Osborn *et al.*, 1997; Barkay *et al.*, 2003). Among them only *Pseudoalteromonas haloplanktis* and some *Caulobacter* strains are considered to be autochthonous marine bacteria due to the requirement of NaCl for growth.

Two marine mercury-resistant isolates were recovered from water samples collected from the Er-Jen River estuary, Tainan, Taiwan. Polyphasic characterization data from this study indicated that the two isolates should be classified as the type strains of two novel species in the genus *Alteromonas*.

Polypeptone–yeast extract–mercury (PYM) broth media used for selective cultivation of mercury-resistant bacteria were made up of two parts. Part I contained 3 g polypeptone (Nihon Seiyaku), 1 g Bacto yeast extract (Difco), 25 g NaCl and 2 g $MgCl_2.6H_2O$ dissolved in 900 ml deionized water and adjusted to pH 8.0. Part II contained 2.7–13.5 mg (approximately 10–50 μmol) $HgCl_2$ dissolved in 100 ml deionized water. The two parts were autoclaved separately and mixed at room temperature. Other culture media used for the present study were as described by Shieh *et al.* (2000), while polypeptone–yeast extract (PY) broth and its derivatives were modified with $MgCl_2.6H_2O$ at 2 g l^{-1} and were adjusted to pH 8.0.

The Er-Jen River is located in south-west Taiwan, and is notorious for severe heavy-metal pollution by some unregulated metal reclamation processing sites at the downstream area since the 1970s. Water samples that had salinities of 14.0–34.6 psu were collected from a shallow estuarine region of the river. Each sample was diluted 10-fold with sterile NaCl/Tris buffer (30 g NaCl and 0.24 g Tris in 1 litre deionized water, pH 8.0). Portions (1 ml) of the water samples and their dilutions (10- to 100-fold) were transferred to culture tubes containing PYM broth (10 ml). All culture tubes were incubated aerobically at 30 °C in the dark for 7 days. Cultures that developed visible turbidity were streaked (one loopful) on PY plate medium. Individual colonies appearing on each of the plates were picked off and purified by successive streaking on PY plates. PY stab cultures of the isolates were maintained at 25 °C under aerobic conditions. Strains AT1^T and AS1^T, two of the isolates deposited in both the Japan Collection of Microorganisms (JCM) and the Bioresource Collection and Research Center (BCRC) as lyophilized cultures, were used for the present study.

Strains AT1^T and AS1^T were grown in PY broth at 30 °C and at the early stationary phase were inoculated (0.1 ml) into tubes containing 10 ml PYM broth with approximately 44 μM $HgCl_2$, in order to determine mercury-resistant growth and mercury-reducing activity. All culture tubes were sealed with rubber stoppers and incubated statically at 30 °C. Bacterial growth was monitored daily according to OD_{600} readings by using a Spectronic Instruments 20D$^+$ spectrophotometer. Before mercury measurement, the converted Hg^0 remaining in each culture was removed by purging for 5 min with a high-purity N_2 gas. Residual mercury was measured after chemical digestion with BrCl by UV oxidation, followed by $NH_2OH.HCl$ pre-reduction and $SnCl_2$ reduction (Bloom & Crecelius, 1983). Total mercury analysis was carried out daily for up to 4 days by an online purge and trap system combined with flow-injection and gold amalgamation pre-concentration techniques, together with cold vapour atomic fluorescence detection (Tseng *et al.*, 2003). Instrument calibration was performed before and during sample analysis by injection of a Hg^0 vapour standard of known mass into the argon gas stream and the bubbler, a gas–liquid separator. The relative standard deviation of replicate samples, a measure of overall precision, was less than 5 % ($n=3$). Recovery from analytical spikes averaged 100 ± 5 % ($n=10$). All experimental steps were in the dark when possible and were in compliance with the ultraclean techniques of Tseng *et al.* (2003) throughout the procedure. All data presented here represent the means of at least three replicates.

Physiological and morphological characteristics of strains AT1^T and AS1^T were determined following the established procedures described in our recent report (Jean *et al.*, 2006a) with modifications and additional tests as described below. Haemolysis was tested by growing cells on blood-agar plates (6.5 % sheep blood; Creative Microbiologicals) that had been spread with sterile NaCl/Tris buffer (0.1 ml). Hydrolysis of chitin was tested by growth of the strains on a modified PY plate medium containing colloidal chitin (Hobel *et al.*, 2005) at 2 g l^{-1}. Besides the cells grown in PY broth, colonies produced on PY plate medium at 20 or 28 °C for 3 days or more were also used for observation of prosthecae and buds via transmission electron microscopy (Bouchotroch *et al.*, 2001; Van Trappen *et al.*, 2004). All the test cultures were incubated aerobically at 30 °C in the dark for 7 days, unless stated otherwise.

Strains AT1^T and AS1^T were cultivated aerobically in PY broth at 30 °C in the dark for 2 days. The cultures were centrifuged to harvest cell mass for analysis. Polar lipids in the cells were extracted, purified and analysed by the methods described by Lin & Shieh (2006). Isoprenoid quinones were extracted, purified and analysed by using an HPLC apparatus equipped with a reversed-phase column (Jean *et al.*, 2006b). Fatty acids in whole cells grown on PY plate medium at 30 °C for 2 days were extracted, saponified and esterified, followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1997). This work was performed at the BCRC, Food Industry Research and Development Institute, Taiwan. Determination of the DNA G+C content by HPLC analysis (Shieh & Liu, 1996) was also performed at the BCRC.

Cells grown in PY broth at 30 °C for 2 days were harvested by centrifugation. Extraction and purification of total genomic DNA from the cells and PCR amplification of

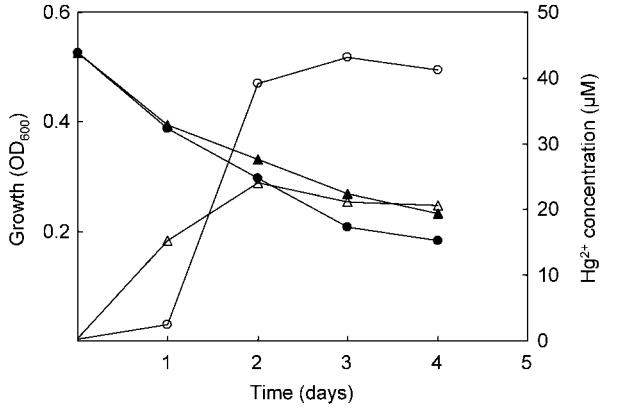

Fig. 1. Changes in OD_{600} (open symbols) and Hg^{2+} concentration (filled symbols) during growth of strains AT1^T (circles) and AS1^T (triangles) in PYM broth.

16S rRNA genes followed the methods described by Jean *et al.* (2006a). Sequencing of the 16S rRNA genes, alignment and comparison of the resulting sequences with reference sequences available in the GenBank database, calculation of distance matrices for the aligned sequences and reconstruction of phylogenetic trees by the neighbour-joining, maximum-parsimony and maximum-likelihood methods

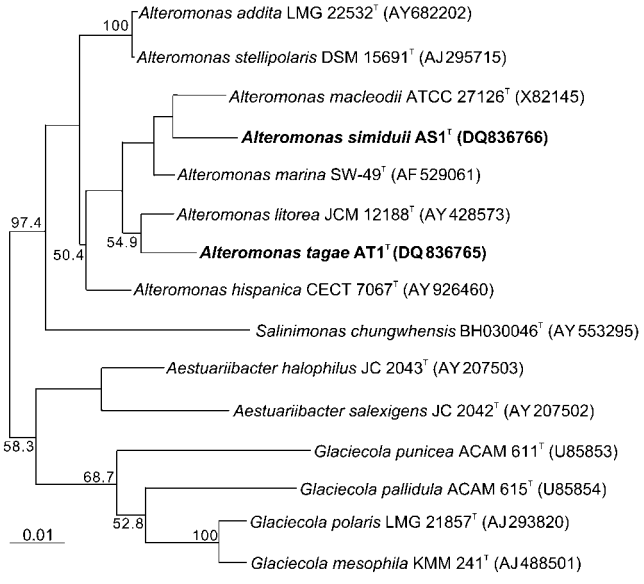

Fig. 2. Unrooted phylogenetic tree derived from neighbour-joining analysis of 16S rRNA gene sequences, showing the relationship between strains AT1^T and AS1^T and recognized *Alteromonas* species, together with related taxa belonging to the family *Alteromonadaceae*. GenBank accession numbers are given in parentheses. Only bootstrap values above 50% are shown at branch nodes (percentages of 1000 replications). Bar, 1% estimated sequence divergence.

Table 1. Cellular fatty acid contents (%) of strains AT1^T and AS1^T and type strains of recognized *Alteromonas* species

Strains: 1, AT1^T (data from this study); 2, AS1^T (this study); 3, *A. addita* R10SW13^T (Ivanova *et al.*, 2005); 4, *A. hispanica* F-32^T (Martínez-Checa *et al.*, 2005); 5, *A. litorea* TF-22^T (Yoon *et al.*, 2004); 6, *A. macleodii* DSM 6062^T (Yoon *et al.*, 2003); 7, *A. marina* SW-47^T (Yoon *et al.*, 2003); 8, *A. stellipolaris* ANT 69a^T (Van Trappen *et al.*, 2004). −, Not detected/not reported; tr, trace amount detected (<1%).

Fatty acid	1	2	3	4	5	6	7	8
$C_{10:0}$ 3-OH	1.5	tr	3.3	1.2	1.4	1.5	1.3	−
$C_{11:0}$	tr	tr	−	−	−	−	−	−
$C_{11:0}$ 3-OH	1.6	tr	2.5	−	1.2	−	1.0	−
iso-$C_{11:0}$ 3-OH	tr	tr	2.2	−	−	−	−	−
$C_{12:0}$	2.9	2.9	1.0	1.8	2.7	2.5	2.9	−
iso-$C_{12:0}$	tr	−	−	−	−	−	−	−
iso-$C_{12:0}$ 3-OH	tr	−	1.1	−	−	−	−	−
$C_{12:0}$ 3-OH	1.2	1.5	1.9	1.1	1.1	1.2	1.1	−
$C_{12:1}$ 3-OH	tr	−	−	−	−	−	−	−
$C_{13:0}$	tr	tr	tr	−	−	−	−	−
iso-$C_{13:0}$	−	tr	−	−	−	−	−	−
anteiso-$C_{13:0}$	tr	tr	−	−	−	−	−	−
$C_{14:0}$	2.4	5.7	2.9	2.1	3.6	2.5	2.6	−
iso-$C_{14:0}$	tr	tr	tr	−	−	−	−	−
$C_{14:0}$ 3-OH	−	−	2.9	−	−	−	−	−
$C_{14:1}$	−	−	1.9	−	−	−	−	−
$C_{15:0}$	−	−	1.8	−	2.1	2.5	2.8	−
$C_{15:0}$ 3-OH	−	−	1.2	−	−	−	−	−
anteiso-$C_{15:0}$	tr	tr	−	−	−	−	−	−
$C_{15:1}\omega6c$	tr	−	−	−	−	−	−	−
$C_{15:1}\omega8c$	1.6	1.5	2.7	−	1.0	1.7	1.1	−
$C_{16:0}$	19.0	22.7	15.2	13.8	20.0	23.8	21.2	12.6
iso-$C_{16:0}$	1.0	1.0	tr	−	−	1.1	−	−
$C_{16:0}$ 3-OH	−	tr	1.4	−	−	−	−	tr
$C_{16:0}$ N alcohol	1.2	tr	−	7.4	5.6	6.6	3.2	tr
$C_{16:1}\omega7c$ alcohol	tr	−	30.1	1.2	5.8	4.3	1.9	tr
$C_{17:0}$	8.2	2.0	2.0	−	2.9	2.6	3.2	−
10-Methyl $C_{17:0}$	tr	tr	−	15.6	4.5	2.9	1.5	−
iso-$C_{17:0}$	−	tr	−	−	−	−	−	−
anteiso-$C_{17:0}$	tr	tr	−	−	−	−	−	−
$C_{17:1}\omega6c$	tr	−	−	−	−	−	−	−
$C_{17:1}\omega8c$	9.4	4.5	4.1	−	3.6	4.3	5.6	9.4
$C_{18:0}$	2.1	tr	1.0	5.9	−	−	−	−
$C_{18:1}\omega7c$	11.7	11.3	11.7	14.3	12.5	9.9	11.8	18.0
$C_{18:1}\omega9c$	tr	−	−	−	−	−	−	−
iso-$C_{18:0}$	tr	tr	7.8	−	−	−	−	−
11-Methyl $C_{18:1}\omega7c$	−	tr	−	−	−	−	−	−
$C_{18:3}\omega6c$ (6,9,12)	−	−	−	5.0	−	−	−	−
$C_{13:0}$ 3-OH/iso-$C_{15:1}$	−	tr	−	−	−	−	−	−
iso-$C_{15:0}$ 2-OH/ $C_{16:1}\omega7c$	22.4	33.7	−	22.1	20.0	24.6	28.5	27.3
iso-$C_{16:1}$ I/$C_{14:0}$ 3-OH	3.9	−	−	2.6	3.0	3.3	3.6	−

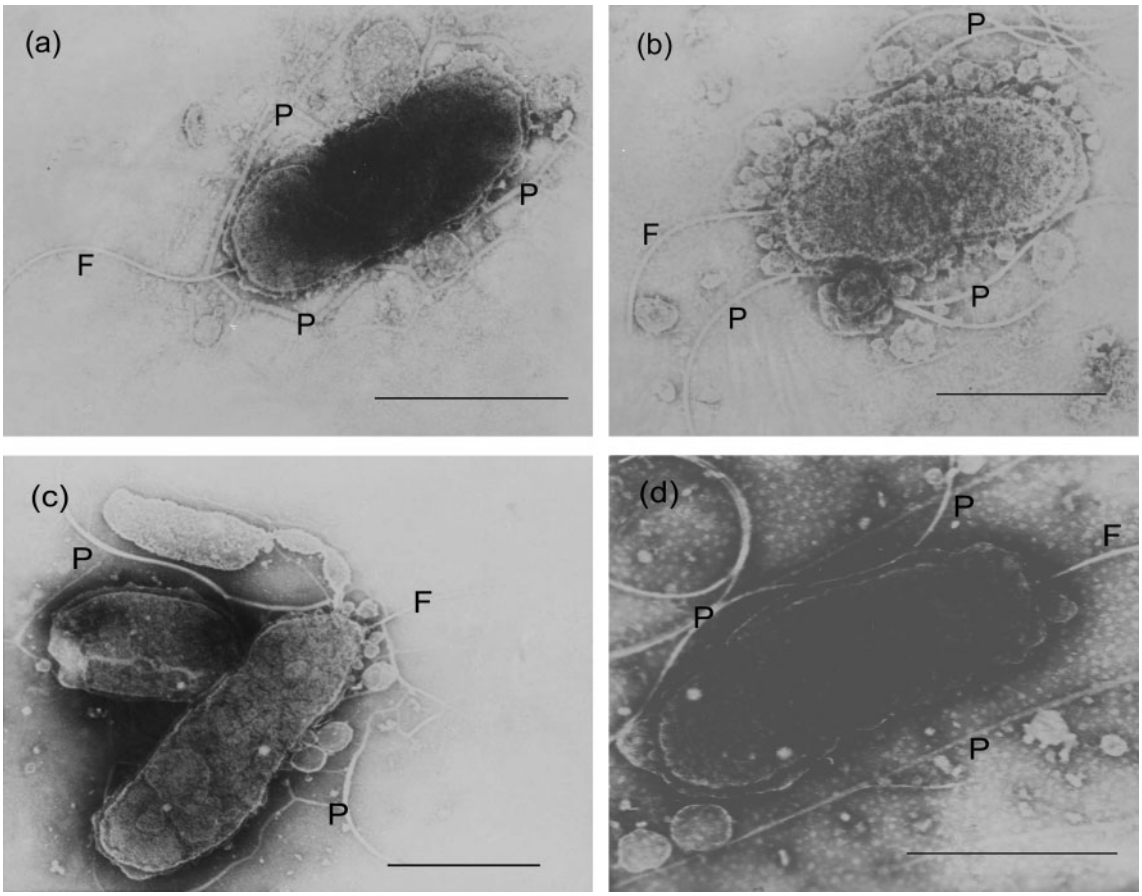

Fig. 3. Electron micrographs of negatively stained preparations of cells of strains AT1^T (a, b) and AS1^T (c, d), showing prosthecae (P), flagella (F) and bleb-like structures. Cells used for analysis were grown on PY plate medium at 20 °C for 4 days (a, c) or in PY broth at 30 °C for 3 days (b, d). Bars, 1 μm.

were performed as described in our recent reports (Shieh *et al.*, 2004; Jean *et al.*, 2006a). The stability of clusters was evaluated by a bootstrap analysis of 1000 replications.

Strains AT1^T and AS1^T grew in PY broth over a pH range of 6–9, and most rapidly at pH 7–8. No growth was observed at pH 5. The two strains grew in PY broth over a temperature range of 15–40 °C, with an optimum at about 30 °C. Only strain AS1^T grew at 10 °C within 15–20 days of incubation. Neither of the strains grew at 4 or 42–45 °C. The two strains were halophilic bacteria that grew well in PY broth at NaCl levels of 0.5–12 % (maximal OD_{600} >0.5), with optimum growth at 2–4 % NaCl. No growth was observed at 0 or 14–15 % NaCl. Strain AT1^T grew at 13 % NaCl within 5–10 days of incubation (maximal OD_{600} >0.3) but strain AS1^T did not; weak growth was observed for strain AS1^T after incubation for 15–20 days (maximal OD_{600} <0.1). Strains AS1^T and AT1^T were mercury-resistant bacteria. They grew significantly in PYM broth containing $HgCl_2$ at an initial concentration of about 44 μM. Mercury-resistant growth was accompanied by a rapid decrease in Hg^{2+} concentration (>20 μM) within 3 days (Fig. 1). Strains AT1^T and AS1^T could have reduced Hg^{2+} to Hg^0 during this growth.

The 16S rRNA gene sequences that had been determined for strains AT1^T and AS1^T comprised 1479 and 1391 nt, respectively. They were aligned and compared with all bacterial sequences available in the GenBank database. The two sequences shared 94.6 % sequence similarity (75 differences out of 1391 nt positions). The signature nucleotides present in the family *Alteromonadaceae* (Ivanova *et al.*, 2004), 304 (A), 734 (A), 736 (T), 770 (T) and 809 (A), were also present in these sequences. Moreover, phylogeny based on neighbour-joining analysis of 16S rRNA gene sequences revealed that the closest neighbours of strains AT1^T and AS1^T were species of the genus *Alteromonas* in the family *Alteromonadaceae* (Fig. 2). Similar results were obtained with the maximum-parsimony and maximum-likelihood algorithms (data not shown). Strains AT1^T and AS1^T showed, respectively, 93.9–95.8 and 95.3–96.8 % 16S rRNA gene sequence similarity to the type strains of the six recognized *Alteromonas* species. The next closest neighbours of strains AT1^T and AS1^T were species of the genera *Aestuariibacter* (90.5–93.5 % sequence similarity) and *Salinimonas* (91.5–91.7 %) in the family *Alteromonadaceae*. No other species shared more than 91 % sequence similarity with the two novel strains.

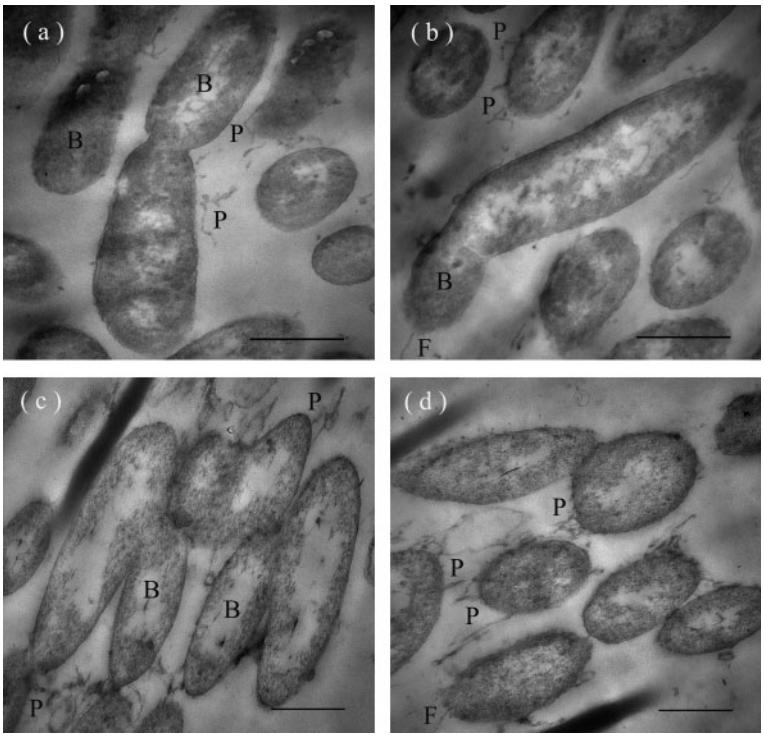

Fig. 4. Electron micrographs of thin-section preparations of cells of strains AT1^T (a, b) and AS1^T (c, d), showing buds (B), prosthecae (P) and flagella (F). Cells used for analysis were grown on PY plate medium at 28 °C for 4 days. Thin-section preparations were stained with 1 % uranyl acetate and 10 % lead citrate. Bars, 0.5 μm.

The DNA G+C contents of strains AT1^T (43.1 mol%) and AS1^T (45.3 mol%) fell within the range of reported values for recognized *Alteromonas* species (43.0–46.4 mol%). The two novel strains, like most other members of the genus *Alteromonas*, contained $C_{16:0}$ (19.0–22.7 %), $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (22.4–33.7 %; the two fatty acids could not be differentiated in the MIDI system) and $C_{18:1}\omega7c$ (11.3–11.7 %) as the major cellular fatty acids. The remaining cellular fatty acids are listed in Table 1. Quantitative differences in the fatty acids can be used to differentiate the two strains from recognized species of the genus *Alteromonas* despite the different cultivation conditions used. The polar lipids of strain AT1^T consisted of phosphatidylethanolamine (46.6 %), phosphatidylglycerol (28.9 %) and sulfolipid (24.5 %), whereas in strain AS1^T they consisted of phosphatidylethanolamine (48.2 %) and phosphatidylglycerol (51.8 %). Some *Alteromonas* species, such as *Alteromonas addita* and *Alteromonas macleodii*, were also found to have phosphatidylethanolamine and phosphatidylglycerol as major polar lipids (Ivanova *et al.*, 2000, 2005). Strains AT1^T and AS1^T contained Q-8 as the predominant isoprenoid quinone (99.9 % for AT1^T and 91.3 % for AS1^T), but only AS1^T contained Q-4 (4.3 %) and Q-6 (4.3 %). Q-8 was detected as the predominant isoprenoid quinone in *Alteromonas hispanica* (96.5 %; Martínez-Checa *et al.*, 2005), *Alteromonas macleodii* (92 %; Yoon *et al.*, 2003), *Alteromonas marina* (94 %; Yoon *et al.*, 2003) and *Alteromonas litorea* (Yoon *et al.*, 2004). However, Q-4 and Q-6 have not been detected in these *Alteromonas* species.

The genus *Alteromonas* has been shown to include prosthecate, budding bacteria (Van Trappen *et al.*, 2004; Martínez-Checa *et al.*, 2005). Both strains AT1^T and AS1^T were also found to produce buds and prosthecae (Figs 3 and 4). They shared many other phenotypic characteristics. The two, however, could be differentiated from each other by different colony types and by different reactions in tests for hydrolysis of casein, lecithin and starch, activity of cystine arylamidase and α-galactosidase, reduction of nitrate to nitrite, growth at 10 °C, growth on D-fructose as a sole carbon and energy source and sensitivity to the vibriostatic agent O/129.

Phylogeny based on 16S rRNA genes and data from chemotaxonomic studies support the establishment of two novel species in the genus *Alteromonas* to accommodate the two novel strains described here. The names *Alteromonas tagae* sp. nov. and *Alteromonas simiduii* sp. nov. are proposed, with AT1^T and AS1^T as the respective type strains. Table 2 shows characteristics that are useful for differentiation between the two novel species and the other recognized species of the genus *Alteromonas*. Detailed characterization data are given below in the species descriptions.

The present study has provided the first evidence that the genus *Alteromonas* includes mercury-resistant species. *Alteromonas tagae* and *Alteromonas simiduii* have thus far been found only in saline estuarine water. These species may readily occur in other saline habitats, given that they can grow over rather wide ranges of temperatures and salinities and do not require organic growth factors.

Table 2. Characteristics that differentiate strains AT1^T and AS1^T from recognized species of the genus *Alteromonas*

Strains: 1, AT1^T (data from this study); 2, AS1^T (this study); 3, *A. addita* R10SW13^T (Ivanova *et al.*, 2005); 4, *A. hispanica* F-32^T (Martínez-Checa *et al.*, 2005); 5, *A. litorea* TF-22^T (Yoon *et al.*, 2004); 6, *A. macleodii* DSM 6062^T (unless indicated, data from Bowman & McMeekin, 2005); 7, *A. marina* SW-47^T (Yoon *et al.*, 2003); 8, *A. stellipolaris* ANT 69a^T (Van Trappen *et al.*, 2004). +, Positive; −, negative; W, weakly positive; ND, no data available. Cells of all strains are Gram-negative rods, motile by means of a polar flagellum, and require NaCl for growth, grow at 15–37 °C and pH 6–9 and are positive for oxidase and catalase.

Characteristic	1	2	3	4	5	6	7	8
Cream-coloured colonies	+	−	−	+	+	−	+	+
Buds/prosthecae	+	+	ND	+	ND	+	+	+
Poly-β-hydroxybutyrate accumulation	+	+	−	+	ND	−	ND	−
Growth at/in:								
4 °C	−	−	+	+	−	−	+	+
10 °C	−	+	+	+	+	+	+	+
40 °C	+	+	−	+	+	+	+	−
42 °C	−	−	−	−	+	ND	+	−
3 % NaCl	+	+	+	−	+	ND	+	+
13 % NaCl	+	W	−	+	+	ND	+	−
15 % NaCl	−	−	−	+	−	ND	+	−
Nitrate reduction to nitrite	−	+	−	−	−	−	−	−
Haemolysis	−	−	+	−	ND	ND	ND	−
Hydrolysis of:								
Agar	−	−	W	−	−	−	−	−
Casein	+	−	ND	+	+	ND	+	ND
Lecithin	+	−	−	−	ND	ND	ND	ND
Starch	+	−	+	+	+	+	+	+
H_2S from cysteine	−	−	−	+	−	ND	−	−
Acid from:								
D-Glucose	−	−	ND	−	−	(+)*	−	−
D-Maltose	−	−	ND	+	+	(+)	−	ND
Sucrose	−	−	ND	−	−	(+)	+	W
D-Trehalose	−	−	ND	−	+	(+)	+	ND
Growth on:								
D-Cellobiose	+	+	+	−	ND	+	ND	+
D-Fructose	−	+	+	−	+	+	+	+
D-Galactose	+	+	−	−	+	+	+	+
D-Glucose	+	+	−	−	ND	+	ND	+
D-Lactose	+	+	+	−	+	+	+	−
D-Mannitol	−	−	−	+	−	+	−	+
L-Serine	+	+	−	−	ND	−	ND	−
L-Lactate	+	+	+	−	−	+	−	−
β-Hydroxybutyrate	+	+	+	ND	ND	−	ND	+
D-Sorbitol	−	−	+	−	−	−	−	−
D-Mannose	−	−	+	−	ND	−	ND	+
D-Ribose	+	+	ND	ND	ND	−	ND	−
D-Melezitose	+	+	ND	−	ND	−	ND	ND
API ZYM test results:								
Cystine arylamidase	−	+	ND	ND	−	−	−	−
α-Galactosidase	−	+	ND	ND	−	−	−	W
O/129 sensitivity (150 μg)	+	−	ND	ND	ND	ND	ND	ND
DNA G+C content (mol%)	43.1	45.3	43.0	46.3	46.0	44.9–46.4	44.0–45.0	43.0–45.0

*Data in parentheses are from Yoon *et al.* (2003).

Description of *Alteromonas tagae* sp. nov.

Alteromonas tagae (ta′gae. N.L. gen. n. *tagae* of Taga, named after Nobuo Taga, a pioneering Japanese marine microbiologist).

Cells are Gram-negative rods (1.2–2.5 × 0.5–0.9 μm) that are motile by means of a single polar flagellum. Colonies produced on PY agar plates at 30 °C for 72–96 h are approximately 2.0–3.5 mm in diameter, cream-coloured, opaque, of low convexity and non-luminescent, with somewhat undulatory edges. Swarming does not occur. Buds and prosthecae are commonly produced on cells grown on PY agar plates at 20 or 28 °C for 3–7 days; these structures can also be observed on cells grown in PY broth at 30 °C for 3–7 days. Extracellular bleb-like structures are produced. Endospores are absent. Chemo-organotroph capable of respiratory, but not fermentative, metabolism. Sodium ions are required for growth; growth occurs at NaCl levels of 0.5–13 %, with an optimum at 2–4 % and no growth at 0 or 14–15 % NaCl. Growth occurs at 15–40 °C, with optimum growth at about 30 °C; no growth at 4–10 or 42–45 °C. Able to grow over a pH range of 6–9 but not at pH 5. Acid is not produced from oxidation or fermentation of the following carbohydrates: D-glucose, D-arabinose, L-arabinose, D-cellobiose, D-galactose, D-lactose, D-maltose, D-mannose, D-melibiose, sucrose, D-trehalose, D-xylose, dulcitol, inositol or D-mannitol. Able to reduce Hg^{2+} to Hg^0. Poly-β-hydroxybutyrate is accumulated as an intracellular reserve product. Negative for denitrification and haemolysis tests. Nitrate is not reduced to nitrite. Indole is not produced from tryptophan. H_2S is not produced from thiosulfate. Oxidase and catalase are present, but arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are absent. Aesculin, alginate, casein, DNA, gelatin, lecithin, starch, and Tweens 20 and 80 are hydrolysed but agar, chitin and urea are not. Able to grow on the following compounds as sole carbon and energy sources: D-cellobiose, D-glucose, D-galactose, D-lactose, D-melezitose, D-melibiose, D-ribose, sucrose, D-trehalose, glycerol, acetate, β-hydroxybutyrate, L-lactate, L-alanine, L-glutamic acid and L-threonine. Unable to grow on the following compounds as sole carbon and energy sources: D-arabinose, L-arabinose, D-fructose, D-mannose, D-xylose, dulcitol, inositol, D-mannitol, D-sorbitol, citrate, fumarate, malonate, tartrate and D-arginine. The following constitutive enzyme activities are detected in API ZYM tests: leucine arylamidase, valine arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), acid phosphatase, alkaline phosphatase and naphthol-AS-BI-phosphohydrolase. Polar lipids comprise phosphatidylethanolamine (46.6 %), phosphatidylglycerol (28.9 %) and sulfolipid (24.5 %). Q-8 is the only isoprenoid quinone (99.9 %) but trace amounts of some others may also occur. Cellular fatty acids present at levels greater than 3 % include $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (22.4 %), $C_{16:0}$ (19.0 %), $C_{18:1}\omega7c$ (11.7 %), $C_{17:1}\omega8c$ (9.4 %), $C_{17:0}$ (8.2 %), and iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH (3.9 %). Sensitive to the vibriostatic agent O/129 at 150 μg. Susceptible to ampicillin (10 μg), chloramphenicol (30 μg), colistin (10 μg), carbenicillin (100 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), neomycin (30 μg), novobiocin (30 μg), polymyxin B (300 U), streptomycin (10 μg) and tetracycline (30 μg), and intermediately susceptible to vancomycin (30 μg). Resistant to cephalothin (30 μg), clindamycin (2 μg), lincomycin (2 μg), oxacillin (1 μg) and penicillin G (10 U). The DNA G+C content is 43.1 mol%.

The type strain, AT1^T (=BCRC 17571^T=JCM 13895^T), was isolated from a water sample collected at the estuary of the Er-Jen River, Tainan, Taiwan.

Description of *Alteromonas simiduii* sp. nov.

Alteromonas simiduii (si.mi′du.i.i. N.L. gen. n. *simiduii* of Simidu, named after Usio Simidu, a Japanese microbiologist, for his work on marine microbiology).

Description is as for the species description of *Alteromonas tagae* with the following differences. Cells are 1.2–2.5 μm long and 0.4–0.8 μm wide. Colonies produced on PY agar plates at 30 °C for 72–96 h are approximately 1–3 mm in diameter, off-white, translucent, umbonate and non-luminescent, with somewhat undulatory edges. Growth occurs in PY broth at 10 °C within 15–20 days incubation. Growth occurs at NaCl levels of 0.5–13 %; growth is slow and weak at 13 % NaCl. Casein, lecithin and starch are not hydrolysed. D-Fructose can be utilized as a sole carbon and energy source for growth. Nitrate is reduced to nitrite. Cystine arylamidase and α-galactosidase are present in API ZYM tests. Polar lipids comprise phosphatidylethanolamine (48.2 %) and phosphatidylglycerol (51.8 %). Isoprenoid quinones comprise Q-8 (91.3 %), Q-4 (4.3 %) and Q-6 (4.3 %). Cellular fatty acids present at levels greater than 3 % include $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (33.7 %), $C_{16:0}$ (22.7 %), $C_{18:1}\omega7c$ (11.3 %), $C_{14:0}$ (5.7 %) and $C_{17:1}\omega8c$ (4.5 %). Resistant to the vibriostatic agent O/129 at 150 μg. Resistant to vancomycin (30 μg). The DNA G+C content is 45.3 mol%.

The type strain, AS1^T (=BCRC 17572^T=JCM 13896^T), was isolated from a water sample collected at the estuary of the Er-Jen River, Tainan, Taiwan.

Acknowledgements

We are grateful to Dr J.-S. Chen for advice and critical reading of the manuscript. This study was supported by grants NSC91-2313-B-002-327, NSC92-2313-B-002-084 and NSC94-2313-B-002-066 from the National Science Council, Taiwan.

References

Barkay, T., Miller, S. M. & Summers, A. O. (2003). Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiol Rev* **27**, 355–384.

Bloom, N. S. & Crecelius, E. A. (1983). Determination of mercury in seawater at sub-nanogram per liter levels. *Mar Chem* **14**, 49–59.

Bouchotroch, S., Quesada, E., del Moral, A., Llamas, I. & Béjar, V. (2001). *Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **51**, 1625–1632.

Bowman, J. P. & McMeekin, T. A. (2005). Genus I. *Alteromonas* Baumann, Baumann, Mandel and Allen 1972, 418, emend. Gauthier, Gauthier and Christen 1995a, 760. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 444–447. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Hobel, C. F. V., Marteinsson, V. T., Hreggvidsson, G. O. & Kristjansson, J. K. (2005). Investigation of the microbial ecology of intertidal hot springs by using diversity analysis of 16S rRNA and chitinase genes. *Appl Environ Microbiol* **71**, 2771–2776.

Ivanova, E. P., Zhukova, N. V., Svetashev, V. I., Gorshkova, N. M., Kurilenko, V. V., Frolova, G. M. & Mikhailov, V. V. (2000). Evaluation of phospholipid and fatty acid compositions as chemotaxonomic markers of *Alteromonas*-like Proteobacteria. *Curr Microbiol* **41**, 341–345.

Ivanova, E. P., Flavier, S. & Christen, R. (2004). 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. *Int J Syst Evol Microbiol* **54**, 1773–1788.

Ivanova, E. P., Bowman, J. P., Lysenko, A. M., Zhukova, N. V., Gorshkova, N. M., Sergeev, A. F. & Mikhailov, V. V. (2005). *Alteromonas addita* sp. nov. *Int J Syst Evol Microbiol* **55**, 1065–1068.

Jean, W. D., Shieh, W. Y. & Chiu, H.-H. (2006a). *Pseudidiomarina taiwanensis* gen. nov., sp. nov., a marine bacterium isolated from shallow coastal water of An-Ping Harbour, Taiwan, and emended description of the family *Idiomarinaceae*. *Int J Syst Evol Microbiol* **56**, 899–905.

Jean, W. D., Chen, J.-S., Lin, Y.-T. & Shieh, W. Y. (2006b). *Bowmanella denitrificans* gen. nov., sp. nov., a denitrifying bacterium isolated from seawater from An-Ping Harbour, Taiwan. *Int J Syst Evol Microbiol* **56**, 2463–2467.

Ji, G., Salzberg, S. P. & Silver, S. (1989). Cell-free mercury volatilization activity from three marine *Caulobacter* strains. *Appl Environ Microbiol* **55**, 523–525.

Lin, Y.-T. & Shieh, W. Y. (2006). *Zobellella denitrificans* gen. nov., sp. nov. and *Zobellella taiwanensis* sp. nov., denitrifying bacteria capable of fermentative metabolism. *Int J Syst Evol Microbiol* **56**, 1209–1215.

Martínez-Checa, F., Bejar, V., Llamas, I., del Moral, A. & Quesada, E. (2005). *Alteromonas hispanica* sp. nov., a polyunsaturated-fatty-acid-producing, halophilic bacterium isolated from Fuente de Piedra, southern Spain. *Int J Syst Evol Microbiol* **55**, 2385–2390.

Muir, D., Braune, B., DeMarch, B., Norstrom, R., Wagemann, R., Lockhart, L., Hargrave, B., Bright, D., Addison, R. & other authors (1999). Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. *Sci Total Environ* **230**, 83–144.

Munthe, J. (1992). Aqueous oxidation of elemental mercury by ozone. *Atmos Environ* **26A**, 1461–1468.

Osborn, A. M., Bruce, K. D., Strike, P. & Ritchie, D. A. (1997). Distribution, diversity, and evolution of the bacterial mercury resistance (mer) operon. *FEMS Microbiol Rev* **19**, 239–262.

Sasser, M. (1997). *Identification of bacteria by gas chromatography of cellular fatty acids*. MIDI Technical Note 101. Newark, DE: MIDI Inc.

Shieh, W. Y. & Liu, C. M. (1996). Denitrification by a novel halophilic fermentative bacterium. *Can J Microbiol* **42**, 507–514.

Shieh, W. Y., Chen, A. L. & Chiu, H. H. (2000). *Vibrio aerogenes* sp. nov., a facultatively anaerobic marine bacterium that ferments glucose with gas production. *Int J Syst Evol Microbiol* **50**, 321–329.

Shieh, W. Y., Lin, Y.-T. & Jean, W. D. (2004). *Pseudovibrio denitrificans* gen. nov., sp. nov., a marine, facultatively anaerobic, fermentative bacterium capable of denitrification. *Int J Syst Evol Microbiol* **54**, 2307–2312.

Silver, S. & Phung, L. T. (1996). Bacterial heavy metal resistance: new surprise. *Annu Rev Microbiol* **50**, 753–789.

Tseng, C.-M., Balcom, P. H., Lamborg, C. H. & Fitzgerald, W. F. (2003). Dissolved elemental mercury investigations in Long Island Sound using on-line Au amalgamation-flow injection analysis. *Environ Sci Technol* **37**, 1183–1188.

Van Trappen, S., Tan, T.-L., Yang, J., Mergaert, J. & Swings, J. (2004). *Alteromonas stellipolaris* sp. nov., a novel, budding, prosthecate bacterium from Antarctic seas, and emended description of the genus *Alteromonas*. *Int J Syst Evol Microbiol* **54**, 1157–1163.

Wagner-Döbler, I., von Canstein, H. F., Li, Y., Timmis, K. N. & Deckwer, W.-D. (2000). Removal of mercury from chemical wastewater by microorganisms in technical scale. *Environ Sci Technol* **34**, 4628–4634.

Yoon, J.-H., Kim, I.-G., Kang, K. H., Oh, T.-K. & Park, Y.-H. (2003). *Alteromonas marina* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 1625–1630.

Yoon, J.-H., Yeo, S.-H., Oh, T.-K. & Park, Y.-H. (2004). *Alteromonas litorea* sp. nov., a slightly halophilic bacterium isolated from an intertidal sediment of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1197–1201.