Aliagarivorans marinus gen. nov., sp. nov. and *Aliagarivorans taiwanensis* sp. nov., facultatively anaerobic marine bacteria capable of agar degradation

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Two agarolytic strains of Gram-negative, heterotrophic, facultatively anaerobic, marine bacteria, designated AAM1^T and AAT1^T, were isolated from seawater samples collected in the shallow coastal region of An-Ping Harbour, Tainan, Taiwan. Cells grown in broth cultures were straight rods that were motile by means of a single polar flagellum. The two isolates required NaCl for growth and grew optimally at about 25–30 °C, in 2–4 % NaCl and at pH 8. They grew aerobically and could achieve anaerobic growth by fermenting D-glucose or other sugars. The major isoprenoid quinone was Q-8 (79.8–92.0 %) and the major cellular fatty acids were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 26.4–35.6 %), $C_{18:1}\omega7c$ (27.1–31.4 %) and $C_{16:0}$ (14.8–16.3 %) in the two strains. Strains AAM1^T and AAT1^T had DNA G+C contents of 52.9 and 52.4 mol%, respectively. The two strains had a 16S rRNA gene sequence similarity of 98.6 % and shared 84.9–92.4 % sequence similarity with the type strains of *Agarivorans albus* (91.2–92.4 %), eight *Alteromonas* species (84.9–87.1 %), two *Aestuariibacter* species (86.0–87.0 %), *Bowmanella denitrificans* (86.1–86.7 %), eight *Glaciecola* species (85.0–87.9 %) and *Salinimonas chungwhensis* (85.9–86.1 %). Despite their high sequence similarity, strains AAM1^T and AAT1^T had a DNA–DNA relatedness value of only 4.5 %. The data obtained from these polyphasic taxonomic studies revealed that the two agarolytic isolates could be classified as representatives of two novel species in a new genus, *Aliagarivorans* gen. nov., with *Aliagarivorans marinus* sp. nov. [type strain is AAM1^T (=BCRC 17888^T=JCM 15522^T)] as the type species and *Aliagarivorans taiwanensis* sp. nov. [type strain is AAT1^T (=BCRC 17889^T=JCM 15537^T)] as a second species.

Agar, a complex polysaccharide extracted from marine red algae, is widely used as a gelling agent for microbiological culture media. Hydrolysis of this refractory material, a property found only among members of the domain '*Bacteria*', is indicated by the development of softening, depressions or, in some cases, complete liquefaction of the agar surrounding colonies of agarolytic bacteria. Marine agarolytic bacteria are ubiquitous in coastal and estuarine regions. They include species of various genera belonging to the phyla '*Bacteroidetes*' (previously known as the *Cytophaga–Flavobacterium–Bacteroides*) (Yoon *et al.*, 2007), '*Proteobacteria*' (Jean *et al.*, 2006a; Shieh *et al.*, 2008) and '*Verrucomicrobia*' (Scheuermayer *et al.*, 2006; Shieh & Jean, 1998).

Two strains of agarolytic bacteria, AAM1^T and AAT1^T, were isolated in our laboratory from seawater samples collected in the shallow coastal region of An-Ping Harbour, Tainan, Taiwan, during a survey of the diversity of marine agarolytic bacteria in Taiwan. Data from the present polyphasic study indicated that the two isolates could be classified as representatives of two different species in a new genus belonging to the class *Gammaproteobacteria*.

An-Ping Harbour is located in the south-west coast of Taiwan. Seawater samples were collected from the shallow coastal regions of this harbour in the morning at low tide. Each sample was decimally diluted to the order of 10^{-3}

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains AAM1^T and AAT1^T are FJ167390 and FJ167391, respectively.

with sterile NaCl-Tris buffer (30 g NaCl and 0.24 g Tris base in 1 l deionized water, pH 8.0). Aliquots (0.1 ml) of the 10-fold dilutions (10^{-1} to 10^{-3}) were spread onto polypeptone-yeast extract (PY) plate medium (Shieh *et al.*, 2000) in triplicate. The plates were incubated at 25 °C in the dark for 7 days under aerobic conditions. Individual colonies that appeared to be agarolytic were picked off and purified by successive streaking on PY plates. Maintenance of the isolates in our laboratory was performed regularly at intervals of 3–4 months by inoculating early stationary phase cultures grown in PY broth into 7/10-strength seawater at a ratio of 1 : 25 (v/v). Maintenance cultures were kept at 20 °C under aerobic conditions. Lyophilized cultures of both strains were also created.

Physiological and morphological characterization of AAM1^T and AAT1^T, including growth and other phenotypic properties, was carried out according to previously described established procedures (Jean *et al.*, 2006b), with modifications and additional tests as described below. Hydrolysis of chitin and cellulose was tested by growth of the strains on modified PY plate media containing either colloidal chitin or cellulose at 2 g l^{-1} (Shieh *et al.*, 2008). Haemolysis was tested as described by Chiu *et al.* (2007). Utilization of various compounds as sole carbon and energy sources for growth was tested in carbohydrate/mineral (CM) media (Shieh *et al.*, 2004) plus various carbon sources. Sugars and sugar alcohols were both provided at 5 g l^{-1}, whereas organic acids and amino acids were each provided at 2 g l^{-1}. All the test cultures were incubated aerobically at 30 °C in the dark for 7 days, unless stated otherwise.

Cells grown in PY broth and on PY plate medium at 30 °C for 7 days were used for analyses of cellular fatty acids, isoprenoid quinones and DNA G+C content according to the methods described by Shieh *et al.* (2008). DNA–DNA hybridization between strains AAM1^{T}and AAT1^T was performed as described by Jean *et al.* (2009).

Cells grown in PY broth at 30 °C for 7 days were harvested by centrifugation. Extraction and purification of total genomic DNA from the cells and PCR amplification of 16S rRNA genes were performed according to previously described methods (Jean *et al.*, 2006b). Sequencing of the 16S rRNA genes, alignment and comparison of the resulting sequences with reference sequences available in GenBank, calculation of distance matrices for the aligned sequences and reconstruction of phylogenetic trees based on the neighbour-joining, maximum-parsimony and maximum-likelihood methods were performed as described by Shieh *et al.* (2004) and Jean *et al.* (2006b). Stability of clusters was evaluated by bootstrap analysis of 1000 resamplings.

Nearly complete 16S rRNA gene sequences of strains AAM1^T and AAT1^T (1461 nt for each) were determined. Preliminary 16S rRNA gene sequence comparisons of the two sequences with those in GenBank revealed that the two novel isolates were members of the class *Gammaproteobacteria* and were rather closely related to each other (98.6 % sequence similarity, 21 differences out of 1461 nt positions) than to strains of any recognized bacterial species (<93 % sequence similarity). Strains AAM1^T and AAT1^T showed highest levels of sequence similarity to the type strains of *Agarivorans albus* (91.2–92.4 %; Kurahashi & Yokota, 2004), eight *Alteromonas* species (84.9–87.1 %; Bowman & McMeekin, 2005; Chiu *et al.*, 2007; Ivanova *et al.*, 2005; Martínez-Checa *et al.*, 2005; Van Trappen *et al.*, 2004a; Yoon *et al.*, 2003, 2004), two *Aestuariibacter* species (86.0–87.0 %; Yi *et al.*, 2004), *Bowmanella denitrificans* (86.1–86.7 %; Jean *et al.*, 2006c), eight *Glaciecola* species (85.0–87.9 %; Baik *et al.*, 2006; Bowman *et al.*, 1998; Matsuyama *et al.*, 2006; Romanenko *et al.*, 2003; Yong *et al.*, 2007; Van Trappen *et al.*, 2004b; Zhang *et al.*, 2006) and *Salinimonas chungwhensis* (85.9–86.1 %; Jeon *et al.*, 2005). The distant relationship between the two novel isolates and these bacteria was also evident in the neighbour-joining tree, in which the two isolates formed a stable monophyletic clade (bootstrap value, 100 %) located next to a sister clade comprising only *Agarivorans albus* strains (Fig. 1). The two isolates and *Agarivorans albus* (strain MKT 106^T plus other reference strains), together with the type strains of various other species belonging to the *Alteromonadaceae* (eight *Alteromonas* species, eight *Glaciecola* species, two *Aestuariibacter* species, *S. chungwhensis* and *B. denitrificans*) formed a suprageneric monophyletic clade (Fig. 1). Similar results were obtained from the maximum-likelihood and maximum-parsimony algorithms (not shown). The low levels of 16S rRNA gene sequence similarity to all recognized bacterial species (<93 %), together with the phylogenetic data from the three tree-making algorithms employed, showed that the two novel isolates could be assigned to a novel genus.

Strains AAM1^T and AAT1^T, like *Agarivorans albus* and species of the genera *Alteromonas*, *Glaciecola*, *Aestuariibacter*, *Salinimonas* and *Bowmanella*, contained summed feature 3 [$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (the two fatty acids could not be differentiated in the MIDI system); 26.4–35.6 % of the total fatty acids], $C_{18:1}\omega7c$ (27.1–31.4 %) and $C_{16:0}$ (14.8–16.3 %) as the major fatty acids (Table 1). However, the fatty acid profiles of the two novel isolates could be distinguished from those of closely related species by differences in the levels of the fatty acids $C_{18:1}\omega7c$ (27.1–31.4 % vs 0–19.4 %), iso-$C_{18:0}$ (1.1–6.1 % vs 0 % to trace amount detected, with the exception of *Alteromonas addita*) and iso-$C_{16:0}$ (2.8–6.5 % vs 0–1.5 %, with the exception of *Agarivorans albus*). Other quantitative differences in the fatty acids that might serve to differentiate the two novel isolates from the relevant species are listed in Table 1. Although strains AAM1^T and AAT1^T contained similar fatty acid profiles, their fatty acid profiles differed in the proportions of iso-$C_{16:0}$ (2.8 % vs 6.5 %), iso-$C_{18:0}$ (1.1 % vs 6.1 %) and summed feature 3 (35.6 % vs 26.4 %). The two novel isolates contained Q-8 as the predominant isoprenoid quinone (92.0 % for strain

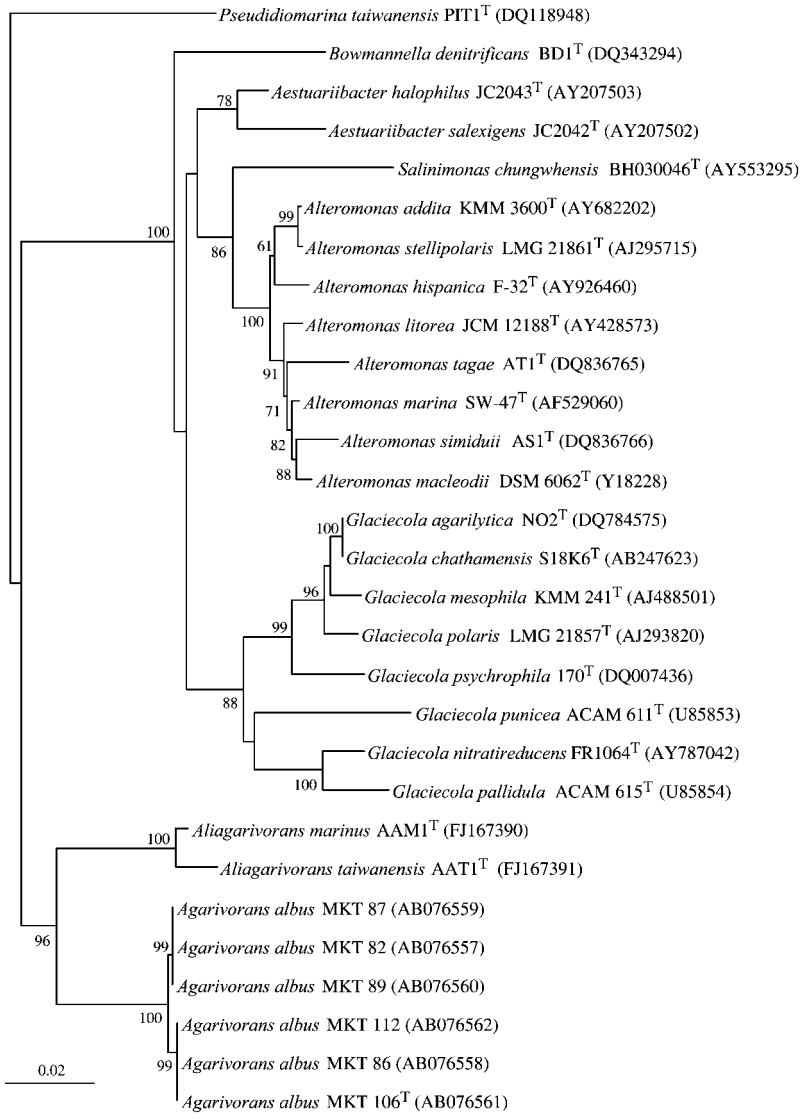

Fig. 1. Neighbour-joining tree showing the phylogenetic relationship between strains AAM1^T and AAT1^T and related bacteria in the class *Gammaproteobacteria*. GenBank accession numbers are given in parentheses. Bootstrap values (percentages of 1000 replicates) above 60 % are shown at branch points. Bar, 2 nt substitution per 100 nt.

AAM1^T and 79.8 % for strain AAT1^T). The remaining isoprenoid quinones included RQ-10 (5.7 % for AAM1^T and 15.6 % for AAT1^T), Q-9 (1.8 % for AAM1^T and 2.0 % for AAT1^T) and MK-8 (0.5 % for AAM1^T and 2.6 % for AAT1^T). They had DNA G+C contents of 52.4–52.9 mol%, which were greater than those of *Agarivorans albus* (48.7–49.7 mol%; Kurahashi & Yokota, 2004), *Alteromonas* species (43.0–46.4 mol%; Bowman & McMeekin, 2005; Chiu *et al.*, 2007; Ivanova *et al.*, 2005; Martínez-Checa *et al.*, 2005; Van Trappen *et al.*, 2004a; Yoon *et al.*, 2003, 2004), *Glaciecola* species (40.0–45.0 mol%; Baik *et al.*, 2006; Bowman *et al.*, 1998; Matsuyama *et al.*, 2006; Romanenko *et al.*, 2003; Yong *et al.*, 2007; Van Trappen *et al.*, 2004b; Zhang *et al.*, 2006), *Aestuariibacter salexigens* (48.0 mol%; Yi *et al.*, 2004), *S. chungwhensis* (48.0 mol%; Jeon *et al.*, 2005) and *B. denitrificans* (50.0 mol%; Jean *et al.*, 2006c). *Aestuariibacter halophilus* was the only species in the *Alteromonadaceae* that had a DNA G+C content that was greater (54.0 mol%; Yi *et al.*, 2004) than those of our isolates. DNA–DNA hybridization showed a DNA relatedness value of 4.5 % between strains AAM1^T and AAT1^T when genomic DNA of the former isolate was used as a probe. The two isolates could be classified as two different genomic species according to this result. Taken together with phylogenetic results based on 16S rRNA gene sequences and analyses of isoprenoid quinones and fatty acids, the two isolates can be classified as representing two distinct species in a novel genus.

Strains AAM1^T and AAT1^T were mesophilic, halophilic and Gram-negative. They produced circular, convex, off-white, opaque and non-luminescent colonies surrounded by shallow depressions when grown on PY plate medium for 4–7 days. Clear yellow haloes formed around the colonies in contrast to the purple–brown background when the agar plates were flooded with iodine/potassium iodide solution (not shown). This indicated diffusion of agarase

Table 1. Cellular fatty acid contents (%) of strains AAM1^T and AAT1^T and members of the genera *Agarivorans*, *Alteromonas*, *Glaciecola*, *Aestuariibacter*, *Salinimonas* and *Bowmanella*

Taxa: 1, strain AAM1^T (data from this study); 2, strain AAT1^T (this study), 3, *Agarivorans albus* MKT 106^T (Kurahashi & Yokota, 2004); 4, *Alteromonas* species [*A. addita* R10SW13^T (Ivanova *et al.*, 2005), *A. hispanica* F-32^T (Martínez-Checa *et al.*, 2005), *A. litorea* TF-22^T (Yoon *et al.*, 2004), *A. macleodii* DSM 6062^T and *A. marina* SW-47^T (Yoon *et al.*, 2003), *A. simiduii* AS1^T and *A. tagae* AT1^T (Chiu *et al.*, 2007), *A. stellipolaris* ANT 69a^T (Van Trappen *et al.*, 2004a)]; 5, *Glaciecola* species [*G. agarilytica* NO2^T (Yong *et al.*, 2007), *G. chathamensis* S18K6^T (Matsuyama *et al.*, 2006), *G. mesophila* KMM 241^T (Romanenko *et al.*, 2003), *G. nitratireducens* FR1064^T (Baik *et al.*, 2006), *G. pallidula* ACAM 615^T and *G. punicea* ACAM 611^T (Bowman *et al.*, 1998), *G. polaris* LMG 21857^T (Van Trappen *et al.*, 2004b), *G. psychrophila* 170^T (Zhang *et al.*, 2006)]; 6, *Aestuariibacter* species [*A. halophilus* JC2043^T and *A. salexigens* JC2042^T (Yi *et al.*, 2004)]; 7, *S. chungwhensis* BH030046^T (Jeon *et al.*, 2005); 8, *B. denitrificans* BD1^T (Jean *et al.*, 2006c). −, Not detected/not reported; tr, trace amount detected (<1 %).

Fatty acid	1	2	3	4	5	6	7	8
$C_{10:0}$ 3-OH	−	−	−	0–3.3	0–2.8	tr–4.6	tr	tr
$C_{11:0}$ 3-OH	−	−	−	0–2.5	0–1.0	tr–1.0	1.3	1.0
$C_{12:0}$	4.9	3.3	6.5	0–2.9	0–4.6	1.8–3.0	3.7	2.5
$C_{12:0}$ 3-OH	−	−	−	0–1.9	0–5.5	tr–1.0	2.5	7.1
$C_{12:1}$ 3-OH	−	−	−	0–tr	0–6.5	1.1–2.2	tr	−
$C_{14:0}$	1.9	1.4	−	0–5.7	1.2–7.0	3.1–3.9	3.1	2.1
$C_{14:1}\omega7c$	−	−	−	−	0–3.7	−	−	−
$C_{15:0}$	−	−	−	0–2.8	tr–3.0	2.4–3.8	1.8	−
$C_{15:1}\omega8c$	−	−	−	0–2.7	0–3.4	tr–1.0	1.1	−
$C_{16:0}$	16.3	14.8	28.7	12.6–23.8	9.4–33.0	15.8–23.9	21.2	21.7
iso-$C_{16:0}$	2.8	6.5	4.4	0–1.1	0–1.5	tr–1.1	tr	−
$C_{16:0}$ N alcohol	−	−	−	0–7.4	−	−	tr	3.7
$C_{16:1}\omega7c$ alcohol	−	−	−	0–5.8	−	tr–2.1	−	2.1
$C_{17:0}$	tr	tr	−	0–8.2	0–5.1	2.8–4.5	3.2	1.6
anteiso-$C_{17:0}$	−	−	−	0–tr	0–2.0	−	tr	−
10-Methyl $C_{17:0}$	−	−	−	0–15.6	−	−	−	3.5
$C_{17:1}\omega8c$	−	tr	−	0–9.4	2.6–9.5	3.1–6.8	3.6	3.8
$C_{18:0}$	−	tr	−	0–5.9	0–1.9	tr–2.9	tr	1.5
iso-$C_{18:0}$	1.1	6.1	−	0–7.8	−	−	−	−
10-Methyl $C_{18:0}$	−	−	−	−	0–1.4	−	−	−
$C_{18:1}\omega6c$	−	−	12.1	−	−	−	−	−
$C_{18:1}\omega7c$	27.1	31.4	15.3	9.9–18.0	0–16.3	11.1–14.0	12.8	19.4
$C_{18:3}\omega6c$	−	−	−	0–5.0	−	−	−	−
Summed feature 2*	6.9	4.5	−	0–3.9	−	1.6–2.0	5.3	−
Summed feature 3*	35.6	26.4	25.5	20.0–33.7	0–60.7	27.2–32.5	31.1	24.1

*Summed features represent groups of two fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2 contains $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I. Summed feature 3 contains $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

out from the colonies and release of reducing compounds during agar hydrolysis. Strains AAM1^T and AAT1^T did not liquefy agar in the plate medium despite their ability to degrade agar. Cells of the strains grown in PY broth appeared to be straight, motile and rod-shaped, with a single polar flagellum, as revealed by TEM (not shown). Strain AAT1^T grew more slowly than AAM1^T; strain AAT1^T was slower to produce colonies on PY plate medium and develop visible turbidity in PY broth. Both strains were facultative anaerobes capable of fermenting cellobiose, dulcitol, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannitol, D-mannose, melibiose, sucrose and D-xylose.

Both strains were susceptible to ampicillin (10 μg), carbenicillin (100 μg), chloramphenicol (30 μg), colistin (10 μg), polymyxin B (300 U) and tetracycline (30 μg), but resistant to clindamycin (2 μg), lincomycin (2 μg), oxacillin (1 μg) and vancomycin (30 μg). In addition, strain AAM1^T was susceptible to erythromycin (15 μg), gentamicin (10 μg), neomycin (30 μg), novobiocin (30 μg) and penicillin G (10 U), intermediately susceptible to kanamycin (30 μg), and resistant to cephalothin (30 μg), nalidixic acid (30 μg) and streptomycin (10 μg). On the other hand, strain AAT1^T reacted differently to these antibiotics. It was susceptible to cephalothin and nalidixic acid, intermediately susceptible to erythromycin, gentamicin, neomycin, novobiocin and streptomycin, and resistant to kanamycin and penicillin G.

Strains AAM1^T and AAT1^T shared many physiological and morphological characteristics. However, they could be

Table 2. Characteristics of strains AAM1^T and AAT1^T that enable them to be distinguished from members of the genera *Agarivorans*, *Alteromonas*, *Glaciecola*, *Aestuariibacter*, *Salinimonas* and *Bowmanella*

Taxa: 1, strain AAM1^T (data from this study); 2, strain AAT1^T (this study), 3, *Agarivorans albus* MKT 106^T and other reference strains (Kurahashi & Yokota, 2004); 4, *Alteromonas* spp. [*A. addita* R10SW13^T (Ivanova *et al.*, 2005), *A. hispanica* F-32^T (Martínez-Checa *et al.*, 2005), *A. litorea* TF-22^T (Yoon *et al.*, 2004), *A. macleodii* DSM 6062^T and *A. marina* SW-47^T (Yoon *et al.*, 2003), *A. simiduii* AS1^T and *A. tagae* AT1^T (Chiu *et al.*, 2007), *A. stellipolaris* ANT 69a^T (Van Trappen *et al.*, 2004a)]; 5, *Glaciecola* spp. [*G. agarilytica* NO2^T (Yong *et al.*, 2007), *G. chathamensis* S18K6^T (Matsuyama *et al.*, 2006), *G. mesophila* KMM 241^T (Romanenko *et al.*, 2003), *G. nitratireducens* FR1064^T (Baik *et al.*, 2006), *G. pallidula* ACAM 615^T and *G. punicea* ACAM 611^T (Bowman *et al.*, 1998), *G. polaris* LMG 21857^T (Van Trappen *et al.*, 2004b), *G. psychrophila* 170^T (Zhang *et al.*, 2006)]; 6, *Aestuariibacter* species [*A. halophilus* JC2043^T and *A. salexigens* JC2042^T (Yi *et al.*, 2004)]; 7, *S. chungwhensis* BH030046^T (Jeon *et al.*, 2005); 8, *B. denitrificans* BD1^T (Jean *et al.*, 2006c). +, Positive; −, negative; W, weakly positive; V, variable among strains; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Fermentation of:								
D-Arabinose	W	−	−	−	−	−	−	−
L-Arabinose	+	−	−	−	−	−	−	−
Cellobiose	+	+	−	−	−	−	−	−
D-Galactose	+	+	−	−	−	−	−	−
D-Glucose	+	+	−	−	−	−	−	−
Raffinose	W	−	−	−	−	−	−	−
Trehalose	+	−	−	−	−	−	−	−
myo-Inositol	W	−	−	−	−	−	−	−
Oxidase	−	−	+	+	+	+	+	+
Nitrate reduction	+	+	+	V	V	+	−	+
Indole production	−	−	ND	− or ND	− or ND	−	ND	+
Poly-β-hydroxybutyrate accumulation	+	+	ND	V	− or ND	−	ND	−
Growth at/in:								
10 °C	−	−	ND	V	V	−	+	+
37 °C	+	+	+	+	V	−	+	+
40 °C	−	−	ND	V	V	+	+	+
0 % NaCl	−	−	−	−	−	−	−	+
1 % NaCl	+	+	ND	V	V	+	−	+
3 % NaCl	+	+	ND	V	+	+	+	+
10 % NaCl	−	−	−	V	V	V	+	+
Hydrolysis of:								
Aesculin	−	−	+	V	V	+	+	−
Casein	−	−	ND	V	− or ND	V	+	+
Gelatin	+	W	−	+	V	+	+	+
Urea	−	−	−	− or ND	− or ND	−	+	+
Agar	+	+	+	V	V	−	−	−
Utilization of:								
D-Fructose	−	−	V	V	V	−	−	+
D-Galactose	+	W	ND	V	V	V	ND	+
D-Glucose	+	−	+	+	V	−	+	+
L-Lactate	−	−	ND	V	V	ND	ND	−
D-Lactose	−	−	ND	V	V	−	−	+
Maltose	+	−	+	+ or ND	V	ND	+	ND
D-Mannitol	+	+	+	V	V	ND	−	−
D-Mannose	−	−	−	V	V	−	ND	−
Sucrose	−	−	−	+	V	V	ND	+
D-Xylose	−	+	−	V	− or ND	−	+	+
DNA G+C content (mol%)	52.9	52.4	48.7–49.7	43.0–46.4	40.0–45.0	48.0–54.0	48.0	50.0

differentiated from each other by different reactions in the tests for fermentation of D-arabinose, L-arabinose, melezitose, raffinose, D-ribose, trehalose, *myo*-inositol and D-sorbitol and utilization of L-arabinose, D-glucose, maltose, raffinose, D-xylose, acetate, fumarate, β-hydroxybutyrate, malate, pyruvate and tartaric acid as sole carbon and energy sources. Both strains were distinguishable from *Agarivorans albus*, the eight *Alteromonas* species studied, the eight *Glaciecola* species studied, the two *Aestuariibacter* species studied, *S. chungwhensis* and *B. denitrificans* in that they were capable of fermentative metabolism and were negative for the oxidase reaction. Production of agarase

also distinguished the strains from these species, except for *Agarivorans albus* (Kurahashi & Yokota, 2004), *Alteromonas addita* (Ivanova *et al.*, 2005), *Glaciecola agarilytica* NO2^T (Yong *et al.*, 2007) and *Glaciecola mesophila* (Romanenko *et al.*, 2003). Other characteristics useful for differentiating strains AAM1^T and AAT1^T from these closely related species are listed in Table 2.

The data obtained from this polyphasic taxonomic study strongly support the establishment of two different novel species in a novel genus. Therefore, AAM1^T and AAT1^T are proposed as the type strains of *Aliagarivorans marinus* sp. nov. and *Aliagarivorans taiwanensis* sp. nov., respectively, of the genus *Aliagarivorans* gen. nov., with *Aliagarivorans marinus* as the type species. Characterization data for *Aliagarivorans marinus* AAM1^T and *Aliagarivorans marinus* AAT1^T are given below in the genus and species descriptions.

Description of *Aliagarivorans* gen. nov.

Aliagarivorans (A.li.a.ga.ri.vo′rans. L. pronoun *alius* other, another; N.L. n. *Agarivorans* a name of a bacterial genus; N.L. masc. n. *Aliagarivorans* the other *Agarivorans*).

Members are heterotrophic, Gram-negative rods belonging to the *Gammaproteobacteria*. Cells grown in broth cultures are motile by means of a single, polar flagellum. Facultative anaerobes capable of both respiratory and fermentative metabolism. Mesophilic, halophilic and agarolytic. Oxidase-negative. Catalase-positive. Major isoprenoid quinone is Q-8. Major cellular fatty acids include $C_{18:1}\omega7c$, summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{16:0}$. The DNA G+C content is approximately 52–53 mol%. The type species is *Aliagarivorans marinus*.

Description of *Aliagarivorans marinus* sp. nov.

Aliagarivorans marinus (ma.ri′nus. L. masc. adj. *marinus* of the sea, marine).

Has the following characteristics in addition to those given for the genus. Cells during late exponential to early stationary phase of growth in broth cultures are straight rods, approximately 1.4–2.0 μm long and 1.0–1.2 μm wide. Colonies produced on marine agar and PY plate medium at 30 °C for 4–7 days are circular (approx. 1–2 mm in diameter), off-white, opaque, convex, non-luminescent and agarolytic; agarolytic activity is indicated by the development of softening and depressions, but not by liquefaction of the agar surrounding the colonies. Swarming does not occur. Able to ferment D-glucose, D-arabinose (weakly), L-arabinose, cellobiose, D-fructose (weakly), D-galactose, D-lactose, maltose, D-mannose, melibiose, melezitose, raffinose (weakly), sucrose (weakly), trehalose, D-xylose, dulcitol, *myo*-inositol (weakly fermentative), D-mannitol and D-sorbitol (weakly) with production of acid, but no gas. Unable to ferment D-ribose. Nitrate is reduced to nitrite, but not further to N_2O or N_2. Poly-β-hydroxybutyrate is accumulated as an intracellular reserve product. Indole is not produced from tryptophan. Growth occurs between 15 and 37 °C, with optimum growth at 25–30 °C; no growth occurs at 4–10 or 40–42 °C. Sodium ions are required for growth; growth occurs at NaCl levels of 1–7 % (w/v), with optimum growth at 2–3 %, and no growth occurs at 0 or 8–10 % NaCl. Able to grow over the pH range 7–9, but not at pH 5–6. Not haemolytic. Agar, alginate and gelatin are hydrolysed, but aesculin, casein, cellulose, chitin, DNA, lecithin, starch, Tween 80 and urea are not. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are absent. The following constitutive enzyme activities are detected in API ZYM tests: leucine arylamidase, esterase (C4), β-galactosidase and naphthol-AS-BI-phosphohydrolase. Able to grow on the following compounds as sole carbon and energy sources: L-arabinose, cellobiose, D-galactose, D-glucose, maltose, D-mannitol, acetate, fumarate, β-hydroxybutyrate, pyruvate, L-alanine, L-aspartate, L-glutamate and L-glutamine. Unable to grow on the following compounds as sole carbon and energy sources: D-arabinose, D-fructose, D-lactose, D-mannose, melibiose, raffinose, L-rhamnose, D-ribose, sucrose, D-xylose, trehalose, salicin, dulcitol, glycerol, *myo*-inositol, D-sorbitol, citrate, lactate, malate, succinate, tartaric acid, L-arginine, L-citrulline, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-ornithine, L-phenylalanine, L-serine, L-threonine, L-tyrosine, L-tryptophan and L-valine.

The type strain is AAM1^T (=BCRC 17888^T=JCM 15522^T), isolated from shallow coastal water from An-Ping Harbour, Tainan, Taiwan. The DNA G+C content of the type strain is 52.9 mol%.

Description of *Aliagarivorans taiwanensis* sp. nov.

Aliagarivorans taiwanensis (tai.wan.en′sis. N.L. masc. adj. *taiwanensis* pertaining to Taiwan, where the type strain was isolated).

Has the following characteristics in addition to those given for the genus. Cells during late exponential to early stationary phase of growth in broth cultures are straight rods, approximately 1.2–2.7 μm long and 0.5–1.2 μm wide. Colonies produced on marine agar and PY plate medium at 30 °C for 4–7 days are circular (approx. 1–2 mm in diameter), off-white, opaque, convex, non-luminescent and agarolytic; agarolytic activity is indicated by the development of softening and depressions, but not by liquefaction of the agar surrounding the colonies. Swarming does not occur. Nitrate is reduced to nitrite, but not further to N_2O or N_2. Poly-β-hydroxybutyrate is accumulated as an intracellular reserve product. Indole is not produced from tryptophan. Growth occurs between 15 and 37 °C, with optimum growth at 25–30 °C; no growth occurs at 4–10 or 40–42 °C. Sodium ions are required for growth; growth occurs at NaCl levels of 1–7 % (w/v), with optimum growth at 2–3 %, and no growth occurs at 0 or 8–10 % NaCl. Able to grow over the pH range 7–9, but not at

pH 5–6. Not haemolytic. Agar, alginate and gelatin (weakly) are hydrolysed, but aesculin, casein, cellulose, chitin, DNA, lecithin, starch, Tween 80 and urea are not. Able to ferment D-galactose (weakly), D-glucose, cellobiose, D-fructose, D-lactose (weakly), maltose, D-mannose, melibiose, sucrose, D-xylose (weakly), dulcitol (weakly) and D-mannitol with production of acid, but no gas. Unable to ferment D-arabinose, L-arabinose, melezitose, raffinose, D-ribose, trehalose, *myo*-inositol or D-sorbitol. Able to grow on the following compounds as sole carbon and energy sources: cellobiose, raffinose, D-xylose, D-mannitol, L-aspartate, L-glutamate, malate, tartaric acid, L-glutamine and L-ornithine. Unable to grow on the following compounds as sole carbon and energy sources: D-arabinose, L-arabinose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, melibiose, L-rhamnose, D-ribose, sucrose, trehalose, salicin, dulcitol, glycerol, *myo*-inositol, D-sorbitol, citrate, acetate, fumarate, β-hydroxybutyrate, lactate, succinate, pyruvate, L-alanine, L-arginine, L-citrulline, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-serine, L-threonine, L-tyrosine, L-tryptophan and L-valine. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are absent. Activities of acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8) (weak reaction), α-galactosidase, β-galactosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase (weak reaction) are present in API ZYM tests.

The type strain is AAT1^T (=BCRC 17889^T=JCM 15537^T), isolated from shallow coastal water from An-Ping Harbour, Tainan, Taiwan. The DNA G+C content of the type strain is 52.4 mol%.

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