Aliidiomarina taiwanensis gen. nov., sp. nov., a marine bacterium isolated from shallow coastal water from Bitou Harbour, Taiwan

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Abstract

A Gram-negative, heterotrophic, aerobic, marine bacterium, designated strain AIT1^T, was isolated from a seawater sample collected in the shallow coastal region of Bitou Harbour, Taipei County, Taiwan. Cells grown in broth cultures were straight to slightly curved rods that were motile by means of a single polar flagellum. Strain AIT1^T required NaCl for growth and grew optimally at 30-40 °C and 1.5-5% NaCl. It grew aerobically and was incapable of anaerobic growth by fermentation of glucose or other carbohydrates. Isoprenoid quinones consisted of Q-8 (95.2 %) and Q-9 (4.8 %). Cellular fatty acids were predominantly iso-branched, including iso-$C_{17:0}$ (26.5 %), iso-$C_{17:1}\omega 9c$ (25.9 %) and iso-$C_{15:0}$ (20.5 %). The DNA G + C content was 51.5 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain AIT1^T formed a distinct lineage within the class *Gammaproteobacteria* and exhibited highest levels of sequence similarity with species of *Idiomarina* in the family *Idiomarinaceae* (91.5-93.9 %). The phylogenetic data, together with those from chemotaxonomic, physiological and morphological characterizations, revealed that the novel isolate could be classified as the representative of a novel genus and species in the family *Idiomarinaceae*, for which the name *Aliidiomarina taiwanensis* gen. nov., sp. nov. is proposed. The type strain is AIT1^T (= JCM 16052^T = BCRC 80035^T = NCCB 100320^T).

Halophilic, aerobic, Gram-negative, rod-shaped bacteria belonging to the family *Idiomarinaceae* (Ivanova *et al*., 2004; Jean *et al*., 2006) the order *Alteromonadales* (Bowman & McMeekin, 2005), class *Gammaproteobacteria*, are unique in containing high levels of iso-branched cellular fatty acids (Ivanova *et al*., 2004). These bacteria are also distinguished from other bacteria of the *Alteromonadales* by their poor ability to use carbohydrates as sole carbon and energy sources (Ivanova *et al*., 2004). Members of the family *Idiomarinaceae* have been defined as possessing signature nucleotides in the 16S rRNA sequences, including 143 (C or A), 662 (A), 682 (A), 830 (T) and 856 (A) (numbering by comparison with *E. coli* sequence AE000471, Jean *et al*., 2006). This family formerly comprised both the genera *Idiomarina* (Ivanova *et al*., 2004) and *Pseudidiomarina* (Jean *et al*., 2006). However, recently the species of *Pseudidiomarina* were re-assigned to *Idiomarina* as proposed by Taborda *et al*. (2009) due to the difficulty in differentiating the two genera by the phenotypic characteristics. The current *Idiomarina*, therefore, has accommodated fourteen species, *Idiomarina abyssalis* (Ivanova *et al*., 2000), *I. baltica* (Brettar *et al*., 2003), *I. fontislapidosi* (Martínez-Cánovas *et al*., 2004), *I. insulisalsae* (Taborda *et al*., 2009), *I. loihiensis* (Donachie *et al*., 2003), *I. ramblicola* (Martínez-Cánovas *et al*., 2004), *I. seosinensis* (Choi & Cho, 2005), *I. zobellii* (Ivanova *et al*., 2000), *I. taiwanensis* (Taborda *et al*., 2009), *I. homiensis* (Taborda *et al*., 2009), *I. marina* (Taborda *et al*., 2009), *I. salinarum* (Taborda *et al*., 2009), *I. sediminum* (Taborda *et al*., 2009) and *I. tainanensis* (Taborda *et al*., 2009); the latter six were formerly belonging to the species of the genus *Pseudidiomarina*. All these bacteria were isolated from saline habitats with a wide range of salinities, such as coastal and oceanic waters, coastal sediments, inland hypersaline wetlands, solar salterns and submarine hydrothermal fluids.

The strain AIT1^{T}, a novel isolate recovered in our laboratory from a seawater sample, was collected in the shallow coastal region of Bitou Harbour, Taipei County, Taiwan, during a survey of diversity of heterotrophic marine bacteria. Polyphasic taxonomic data obtained in this study have indicated that this isolate may represent a novel species and genus in the family *Idiomarinaceae*. We propose that the novel taxon represented by strain AIT1^{T} be named *Aliidiomarina taiwanensis* gen. nov., sp. nov.

Bitou Harbour is located on the north coast of Taiwan. The seawater sample was diluted 10-fold 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 decimally diluted samples (10^{-1} to 10^{-3}) were spread on polypeptone-yeast (PY) plate medium (Shieh *et al*., 2000) in triplicate. The plates were incubated at 30 ºC in the dark for 7 days under aerobic

conditions. Individual colonies appearing on the plates were picked off and purified by successive streaking on PY plates. PY stab cultures of the isolates were maintained at 25 ºC under aerobic conditions. One of the isolates, designated strain AIT1^T, was used in the present study. It has been deposited in the Bioresource Collection and Research Center (BCRC), the Japan Collection of Microorganisms (JCM) and the Netherlands Culture Collection of Bacteria (NCCB) as lyophilized cultures.

Growth and other phenotypic properties used for the morphological and physiological characterization of strain AIT1^T were carried out following the established procedures described by Jean *et al*. (2006). Cells grown in PY broth or on PY plate medium at 30 ºC for 3 days were used for analyses of cellular fatty acids, isoprenoid quinones and DNA base composition according to the methods described by Shieh *et al*. (2008). However, the HPLC apparatus used for analysis of isoprenoid quinones was equipped with a column of Inertsil ODS-2 (1.5 × 250 mm, GL Science) instead of Waters model $5C_{18}$-AR-II.

Multivariate analysis using the PC-ORD version 5.1 software (McCune & Mefford, 1999) was performed to analyse the similarity of cellular fatty acids of strain AIT1^T and type strains of recognized species in the family *Idiomarinaceae*. The resulting matrix of similarities was clustered by hierarchical agglomerative clustering with group-average linkage (McCune & Mefford, 1999). Statistical analysis of the similarity between strains was based on the distance and similarity matrices of the cellular fatty acids of each strain.

Cells grown in PY broth at 30 °C for 3 days were harvested by centrifugation. Extraction and purification of total genomic DNA from the cells and PCR amplification of the 16S rRNA gene were performed according to the methods described previously (Jean *et al*., 2006). Sequencing of the 16S rRNA gene, alignment and comparison of the resultant sequences with the 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 carried out by the procedures as described by Shieh *et al*. (2004) and Jean *et al*.(2006). Stability of clusters was evaluated by bootstrap analysis of 1000 resamplings.

The nearly complete 16S rRNA gene sequence (1441nt) of strain AIT1^T was determined. Preliminary 16S rRNA gene sequence comparisons with those in GenBank indicated that strain AIT1^T belonged to the order *Alteromonadales* of the

class *Gammaproteobacteria*. Based on the 16S rRNA gene sequence similarity, this novel isolate showed no apparent relationship with other bacteria; no more than 94.0 % of 16S rRNA gene sequence similarities was found for the strain AIT1 compared to all other bacteria with valid published names. The highest sequence similarities found were observed with the type strains of *Idiomarina* spp. (91.5-93.9 %) in the family *Idiomarinaceae* (Table 1). The distant relationship between strain AIT1^T and other bacteria was also evident in the neighbour-joining tree; strain AIT1^T may represent a novel lineage in the tree, in which this isolate was an outgroup with respect to the *Idiomarinaceae* clade that contained two subclades, phylotypes I and II (Fig. 1). Similar results were also observed in the phylogenetic trees which were reconstructed by using maximum-parsimony and maximum-likelihood algorithms (data not shown). Bacterial species that were not members of the family *Idiomarinaceae* had sequence similarities of less than 90 % with strain AIT1^T. With the phylogenetic data from the three tree-making algorithms mentioned above and the low levels of 16S rRNA gene sequence similarity to all recognized bacterial species (< 94.0 %), it was suggested that strain AIT1^T could be classified as the representative of a novel genus in the family *Idiomarinaceae*.

Strain AIT1^T has the signature nucleotides, 143 (A), 662 (A), 682 (A), 830 (T) and 856 (A), which were distinctive for the family *Idiomarinaceae* (Ivanova *et al*., 2004; Jean *et al*., 2006). However, the strain also has the unique nucleotides 240 (A), 286 (T), 762 (A), 811 (T), 1336 (A) and 1439 (G), distinguished from other *Idiomarinaceae* spp. that have G, C, T, C, C and T/A at these positions, respectively (Table 2). These unique nucleotides may be considered signature nucleotides of a novel genus in the family *Idiomarinaceae* as represented by strain AIT1^T. Each of the phylotypes I and II was also detected to have specific nucleotides among the family *Idiomarinaceae*, including 143 (C), 206 (C), 213 (G), 241 (G), 285 (C), 653 (A), 658 (C), 748 (C) and 1439 (T) in phylotype I and 1256 (A), 1279 (G), 1286 (A) and 1439 (A) in phylotype II (Table 2). The results from detection of the phylotype-specific nucleotide, together with clear phylogenetic divergence (Fig. 1) and low levels of 16S rRNA gene sequence similarity between species of the phylotypes I and II (92.7-95.7 %, Table 1) were consistent with the previous classification that members of phylotypes I and II would belong to the genera *Idiomarina* and *Pseudidiomarina*, respectively (Jean *et al*., 2009). However, species of the *Pseudidiomarina* were recently re-classified to the genus *Idiomarina* due to the difficulty in differentiating the two genera by phenotypic characteristics (Taborda *et al*., 2009).

Strain AIT1^T had a DNA G + C content of 51.5 mol%, lower than *Idiomarina*

salinaum (53.9 mol%), but higher than other *Idiomarinaceae* spp. (45.0-50.4 mol%) (Table 3). This isolate contained Q-8 as the predominant isoprenoid quinone (96.3 %) and Q-9 as a minor one (3.7 %). Some *Idiomarinaceae* spp. were also detected containing Q-8 as the predominant isoprenoid quinone while they contained Q-7 but not Q-9 as the minor quinone (Table 3). Strain AIT1^T, like other *Idiomarinaceae* spp., contained $C_{17:0}$ iso, $C_{15:0}$ iso and $C_{17:1}$ iso ω9*c* as the major cellular fatty acids. The total content of ~~summed~~ $C_{17:0}$ iso and $C_{17:1}$ iso ω9*c*, however, was much more abundant in this isolate than in other *Idiomarinaceae* spp. (52.4% vs. 17.8-36.7%) (Tables 3 & S1). Detection of $C_{15:0}$ iso 3-OH (2.2 %) coupled with absence of $C_{13:0}$ iso/$C_{13:0}$ iso 3-OH also distinguished this isolate from other *Idiomarinaceae* spp. (Tables 3 & S1). Moreover, hierarchical agglomerative clustering with group-average linkage of the resulting matrix of similarities of the cellular fatty acids further differentiated strain AIT1^T from other species of *Idiomarinaceae*. Finally, the cluster analysis clearly demonstrated that strain AIT1^T was an outgroup with respect to the group comprising the recognized species of *Idiomarinaceae* (Fig. 2). The group of the recognized *Idiomarinaceae* species could be divided into four subgroups, one of which consisted of species of phylotype I and the other three groups consisted of species of phylotype II (Fig. 2).

Strain AIT1^T was mesophilic, halophilic and Gram-negative. It produced light brown, circular, convex and non-luminescent, colonies on PY plate medium. Carbohydrate fermentation tests in polypeptone-yeast extract-carbohydrate (PYC) stab media (Shieh *et al*., 2000) showed that the strain did not ferment any of the test carbohydrates, D-arabinose, L-arabinose, D-cellobiose, dulcitol, D-fructose, D-galactose, D-glucose, *myo*-inositol, D-lactose, D-maltose, D-mannitol, D-mannose, D-melezitose, D-melibiose, D-raffinose, D-ribose, D-sorbitol, D-sucrose, D-trehalose and D-xylose. No growth was observed in any of the CM media which contained glucose or other carbohydrates as sole carbon and energy sources, although these media have been shown to support the growth of various other marine bacteria (Shieh *et al*., 2003, 2004). Endospores were not found. Neither sporulation nor endospore-like structures were ever observed or confirmed by phase-contrast microscopy or spore staining. PHB-like granules were not found as intracellular reserve products.

Strain AIT1^T was susceptible to carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol (30 μg), colistin (10 μg), erythromycin (15 μg), gentamicin (10 μg), nalidixic acid (30 μg), novobiocin (30 μg), penicillin G (10 U), polymyxin B (300 U) and tetracycline (30 μg), intermediately susceptible to ampicillin (10 μg), kanamycin (30 μg), neomycin (30 μg) and streptomycin (10 μg), and resistant to oxacillin (1 μg)

and vancomycin (30 µg).

The negative reactions for alkaline phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase in the API ZYM tests allowed strain AIT1^T to be distinguished from the recognized species of *Idiomarinaceae*, except that no comparative data were available for *Idiomarina fontislapidosi* and *Idiomarina homiensis*. Additional characteristics useful for distinguishing strain AIT1^T from other *Idiomarinaceae* spp. are listed in Table 3.

Our polyphasic taxonomic data indicate that strain AIT1^T may represent a novel species and genus in the family *Idiomarinaceae* for which the name *Aliidiomarina taiwanensis* gen. nov., sp. nov. is proposed.

Description of *Aliidiomarina* gen. nov.

Aliidiomarina (A.li.i.di.o.ma.ri.na. L. pronoun. *alius* other, another; N.L. n. *Idiomarina* a name of a bacterial genus; N.L. fem. n. *Aliidiomarina* the other *Idiomarina*).

Members are heterotrophic, Gram-negative rods belonging to the family *Idiomarinaceae* in the class *Gammaproteobacteria*. Cells grown in broth cultures are motile by means of a single, polar flagellum. Chemo-organotrophs capable of respiratory, but not fermentative metabolism. Mesophilic and halophilic. Oxidase- and catalase-positive. Major cellular fatty acids are iso-branched, including $C_{17:0}$ iso, $C_{17:1}$ iso ω9*c* and $C_{15:0}$ iso. Minor cellular fatty acids include $C_{15:0}$ iso 3-OH but not $C_{13:0}$ or $C_{13:0}$ iso 3-OH. Q-8 is the major isoprenoid quinone and Q-9 is a minor one. The genus-specific nucleotide positions in the 16S rRNA genes (numbering by comparison with *E. coli* sequence AE000471) of the family *Idiomarinaceae* include the following: 240 (A), 286 (T), 762 (A), 811 (T), 1336 (A) and 1439 (G). The DNA G + C content is approximately 51-52 mol%. The type species is *Aliidiomarina taiwanensis*.

Description of *Aliidiomarina taiwanensis* sp. nov.

Aliidiomarina taiwanensis (tai.wan.en'sis. NL. fem. adj. *taiwanensis* pertaining to Taiwan, where the type strain was isolated).

Has the following characteristics in addition to those given for the genus. Cells are

Gram-negative and rod-shaped, approximately 1.5-2.8 μm long and 0.5-0.8 μm wide, and motile by means of a single, polar flagellum. Colonies produced on PY agar plates at 30 °C for 7 days are approximately 4-6 mm in diameter, light brown, circular, convex and non-luminescent, with entire edges. Endospores are absent. Poly-β-hydroxybutyrate (PHB) is not accumulated as an intracellular reserve product. Nitrate is reduced to nitrite but not further to N_2O or N_2. Do not grow anaerobically by fermenting glucose or other carbohydrates as substrates. NaCl is required for growth; growth occurs at 0.5-10 %, with optimum growth at 1.5-5 %; does not grow at 0 or 12.5-15% NaCl. Growth occurs at 4-45 °C, with optimum growth at 30-40 °C; does not grow at 50 °C. Growth occurs at pH values between 7 and 9; does not grow at pH 6 or pH 10. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are absent. Indole is produced. H_2S is not produced from thiosulphate. DNA and gelatin are hydrolysed, but aesculin, agar, casein, starch, Tween 80 and urea are not. Only the following constitutive enzyme activities are detected in API ZYM tests: acid phosphatase, valine arylamidase, cystine arylamidase, α-chymotrypsin, naphthol-phosphohydrolase and trypsin. N-Acetylglucosamine, amidone, L-arabinose, erythritol, D-fructose, L-fucose, D-galactose, D-glucose, glycerol, glycogen, D-maltose, D-mannitol, D-mannose, potassium gluconate, D-ribose, D-sucrose and D-trehalose are oxidized in API 50CH tests. Acetic acid, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-asparagine, L-aspartic acid, bromosuccinic acid, dextrin, D-fructose, L-fucose, α-D-glucose, D-glucose-6-phosphate, D-gluconic acid, D-glucosaminic acid, L-glutamic acid, glycogen, glycyl-L-aspartic acid, L-histidine, ρ-hydroxyphenylacetic acid, inosine, D,L-lactic acid, maltose, D-mannitol, D-mannose, D-psicose, pyruvic acid methyl ester, L-serine, sucrose, D-trehalose, thymidine, turanose and uridine are oxidized in Biolog GN2 tests.

The type strain, AIT1^T (= JCM 16052^T = BCRC 80035^T = NCCB 100320^T), was isolated from shallow coastal seawater of Bitou Harbour, Taipei County, Taiwan. It has a DNA G + C content of 51.5 mol%.

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Tables & Figures

Table 1.Values of percent similarity and evolutionary distance (K_{nuc}) of the 16S rRNA gene sequences of strain AIT1^T and type strains of recognized *Idiomarinaceae* species

Table 2. Signature nucleotide positions detected in the 16S rRNA genes of strain AIT1^T and the two 16S rRNA gene groups (phylotypes I and II) in the family *Idiomarinaceae*

Signature nucleotide positions for strain AIT1^T and each of the two phylotypes are shown in bold.

Table 3. Characteristics useful for differentiating strain AIT1^T from recognized *Idiomarinaceae* species.

+, Positive; -, negative/not detected/not reported/trace amount detected (< 1 %); ND, no data available.

Table S1. Cellular fatty acids of strain AIT1^T and type strains of recognized *Idiomarinaceae* species.

-, Not detected/ trace amount detected (< 1 %)/not reported.

Figure 1. Unrooted phylogenetic tree derived from a neighbour-joining analysis of 16S rRNA gene sequences, showing the relationship between strains AIT1^T and type strains of recognized *Idiomarinaceae* species, together with other related taxa belonging to the *Gammaproteobacteria*. GenBank accession numbers are given in parentheses. Only bootstrap values greater than 70% are shown at branch nodes (based on 1000 replications). Bar, 0.01 substitutions per nucleotide position.

Figure2. Cluster analysis of cellular fatty acids of strain AIT1^T and type strains of recognized *Idiomarinaceae* species. Data from Table S1 were used for the cluster analysis.

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