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Aliidiomarina quisquiliarum sp. nov., isolated from landfill leachate

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Abstract

Bacterial strain Y-6^T, isolated from a landfill site in Yiwu, PR China, was characterized using a polyphasic taxonomy approach. Cells were Gram-stain-negative, aerobic, rod-shaped, motile by means of a single polar flagellum and formed pale beige colonies. Strain Y-6^T grew at 4–40 °C (optimal at 30–37 °C), pH 6.5–9.5 (optimal at pH 7.2–8.5) and in the presence of 0.5–10.0% (w/v) NaCl (optimal at 1.0–3.0%). Phylogenetic analysis revealed that strain Y-6^T was a member of the genus *Aliidiomarina* and closely related to *Aliidiomarina taiwanensis* MCCC 1A06493^T with a 16S rRNA sequence similarity of 98.2%. The major cellular fatty acids of the isolate were iso-$C_{15:0}$, $C_{16:0}$, iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}$ $\omega 9c$ and/or 10-methyl-$C_{16:0}$). Q-8 was the predominant ubiquinone. The major polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, aminoglycophospholipid, aminophospholipid, phospholipid, three glycolipids and two unknown lipids. The genomic DNA G+C content was 46.6mol%. The digital DNA–DNA hybridization value between Y-6^T and A. taiwanensis MCCC 1A06493^T was 18.3%. Strain Y-6^T had an average nucleotide identity value of 74.09% with A. taiwanensis MCCC 1A06493^T. Results from the polyphasic taxonomy study support the conclusion that strain Y-6^T represents a novel *Aliidiomarina* species, for which the name *Aliidiomarina quisquiliarum* sp.nov. is proposed. The type strain is Y-6^T (=MCCC 1K06228^T=KCTC 82676^T).

INTRODUCTION

The genus *Aliidiomarina*, a member of the family *Idiomarinaceae*, was first described by Huang *et al.* [1]. Chiu *et al.* subsequently emended the description and proposed to transfer *Idiomarina maris* to this genus [2]. At the time of writing, the genus comprised twelve recognized species (https://lpsn.dsmz.de/). These species were isolated from various habitats such as seawater, saline lake, saline-alkaline soil, sediment and wetland [1–11]. The members of this family are Gram-stain-negative, mesophilic, aerobic or facultatively anaerobic rods, require sodium ions for growth and show a poor ability to use carbohydrates as sole carbon and energy sources [12]. The major cellular fatty acids present are iso-$C_{15:0}$, iso-$C_{17:0}$ and summed feature 9 (comprising iso-$C_{17:1}$ $\omega 9c$ and/or 10-methyl-$C_{16:0}$). The major isoprenoid quinone is ubiquinone 8 (Q-8). The DNA G+C content ranges from 45 to 55mol% [1–11]. This article describes a strain isolated from a landfill site and which is considered to represent a novel species of the genus *Aliidiomarina*.

ISOLATION AND ECOLOGY

A landfill leachate sample was collected from a landfill (Tashan landfill) in Yiwu (29° 12′ N, 120° 05′ E), Zhejiang Province, PR China, in the spring of 2021 and stored at 4°C until use. Strain Y-6^T was isolated from the leachate sample using the serial dilution and plate screening method on modified ZoBell 2216E [13]. A total of 36 isolates belonging to 14 bacterial species were obtained on modified ZoBell agar plates, of which Y-6^T was unique. The modified ZoBell 2216E medium contained (per litre distilled

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Keywords: *Aliidiomarina*; landfill; leachate; draft genome; phylogenetic analysis.
Abbreviations: ANI, average nucleotide identity; APGL, aminoglycophospholipid; APL, aminophospholipid; dDDH, digital DNA–DNA hybridization; DPG, diphosphatidylglycerol; GBDP, genome BLAST distance phylogeny; GL, glycolipid; L, lipid; MA, marine agar; MB, marine broth; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Y-6^T is OL989209. The GenBank/EMBL/DDBJ accession number for the draft genome sequence of strain Y-6^T is JAMFTN000000000.
Five supplementary figures and two supplementary tables are available with the online version of this article.

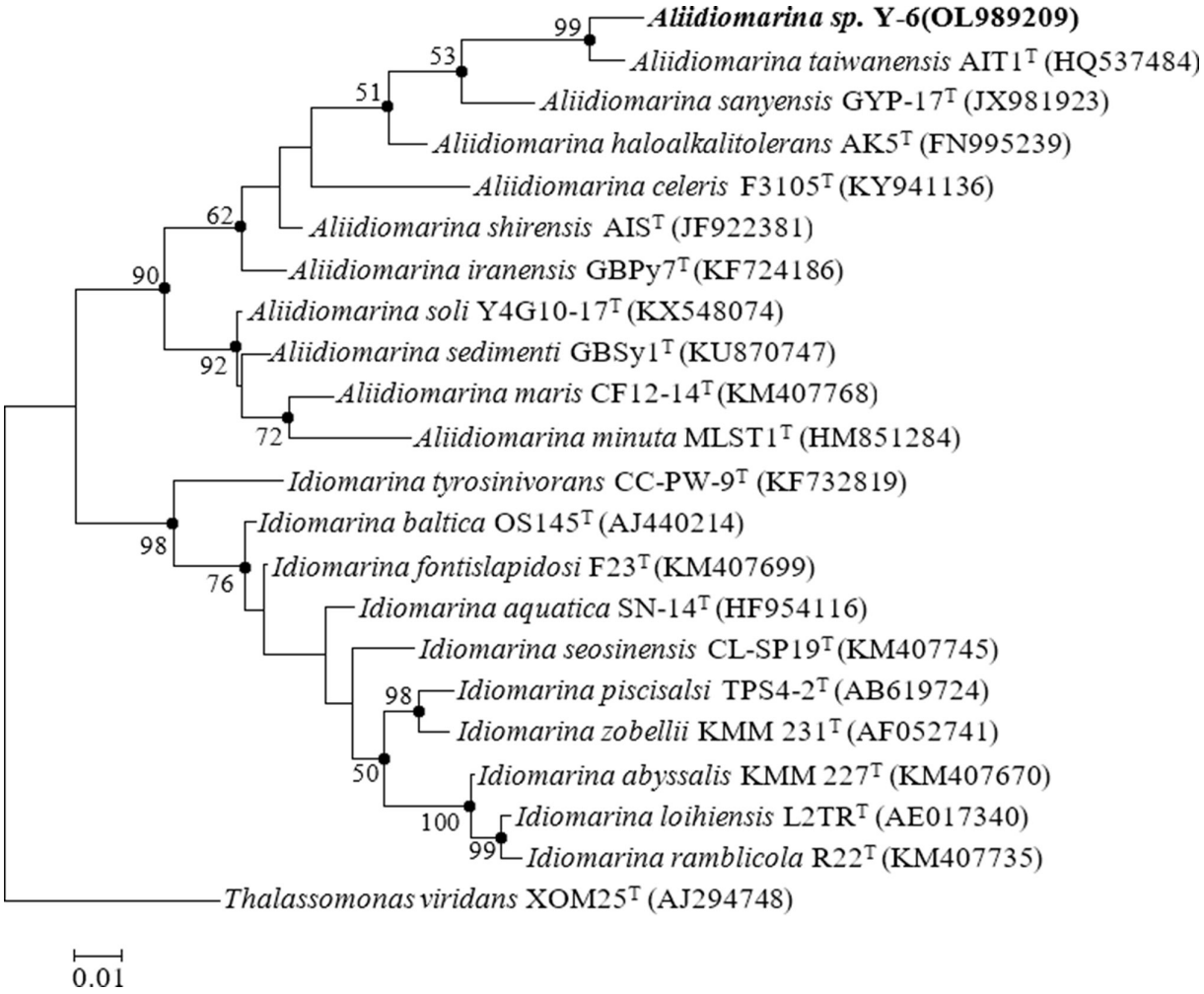

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationships between Y-6^T and related taxa. Bootstrap were expressed higher than 50% at the branch points. Filled circles indicate branches that were recovered with all three methods (maximum-likelihood, neighbour-joining and maximum-parsimony). Bar, 0.01 sequence divergence. *Thalassomonas viridans* XOM25^T (AJ294748) was used as an outgroup. The neighbour-joining and maximum-parsimony trees are shown in Fig. S4 and Fig. S5, respectively.

water): 0.1 g yeast extract, 0.5 g peptone, 0.1 g ferric citrate, 19.45 g NaCl, 8.8 g $MgCl_2{\cdot}6H_2O$, 1.8 g $CaCl_2{\cdot}2H_2O$, 0.55 g KCl, 0.16 g $NaHCO_3$, 3.24 g Na_2SO_4, 0.08 g KBr, 34 mg $SrCl_2$, 22 mg H_3BO_4, 4 mg $NaSiO_4$, 2.4 mg NaF, 1.6 mg NH_4NO_3 and 8 mg Na_2HPO_4, pH 7.4 adjusted with NaOH. Strain Y-6^T was routinely cultured on MA (marine 2216 agar; DB) and preserved at −80 °C in MB (marine 2216 broth; DB) supplemented with 20% (v/v) glycerol for further study.

16S rRNA GENE PHYLOGENY

Genomic DNA was extracted from strains Y-6^T using a Bacterial Genome DNA Rapid Extraction Kit (Dongsheng Biotech). The 16S rRNA gene of strain Y-6^T was amplified with universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGACTTAACCCCAATCGC-3′) [14]. The partial 16S rRNA gene sequence (1508 bp) obtained in this study was preliminarily analysed by the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzBioCloud webserver (www.ezbiocloud.net) [15], and the closely related sequences were selected as references from the list of hits with valid names. Phylogenetic trees were reconstructed using MEGA version 7.0 [16] by the neighbour-joining (NJ) [17], maximum-parsimony (MP) [18] and maximum-likelihood (ML) [19] methods. Strain Y-6^T showed the highest 16S rRNA gene sequence similarity to *Aliidiomarina taiwanensis* MCCC 1A06493^T (98.2% sequence similarity), *Aliidiomarina sanyensis* KCTC 32218^T (95.4%), *Aliidiomarina iranensis* IBRC-M 10763^T (95. 2%) and *Aliidiomarina soli* KCTC 52381^T (94.7%) according to EzBioCloud analysis. The ML phylogenetic tree based on 16S rRNA gene sequences showed that strain Y-6^T formed a distinct cluster with *A. taiwanensis* MCCC 1A06493^T in the genus *Aliidiomarina* (Fig. 1). Similar results were obtained using the NJ and MP algorithms. Strain Y-6^T was likely to represent a novel

Table 1. Differential physiological and biochemical characteristics of strain Y-6^T and its most closely related species

Strains: 1, Y-6^T; 2, *Aliidiomarina taiwanensis* MCCC 1A06493^T; 3, *Aliidiomarina sanyensis* KCTC 32218^T; 4, *Aliidiomarina haloalkalitolerans* MTCC 11064^T. Unless stated otherwise, all data were obtained from this study under identical growth conditions. +, Positive; –, negative; W, weakly positive; ND, no data available; R resistant; S sensitive; M intermediately susceptible.

Characteristic	1	2	3†	4‡
Isolation source	Landfill leachate	Seawater*	Marine spirulina culture pond	Seawater
Cell size (μm)	0.7–1.1×1.5–2.1	0.4–0.8×1.5–3.0*	0.39–0.55×0.74–2.5	0.8–1.0×1.0–1.5
Colony colour	Beige	Light brown*	Dark brown	Creamish
Temperature range for growth (°C)	4–40	4–40*	10–45	10–50
Optimal temperature for growth (°C)	30–37	30–40*	30	30–37
NaCl range for growth (%)	0.5–10	0.5–10*	1–10	0.5–12
Optimal NaCl for growth (%)	1.0–3.0	1.5–5.0*	3–7	0.5–5
pH range for growth	6.5–9.5	7–9*	7–9	7–11
Optimal pH for growth	7.2–8.5	8*	8	8–10
Oxidase	+	+	–	+
Degradation of:				
Gelatin(protease)	+	–	–	+
Tween 20	W	+	+	+
Tween 80	+	+	+	–
API ZYM tests:				
Lipase(C14),	+	–	ND	+
API 50 CH tests:				
D-Glucose	+	+	–	–
D-Mannose	+	+	+	–
D-Fructose	+	+	–	–
Lactose	+	+	–	–
Maltose	W	+	–	–
D-Galactose	W	+	+	–
D-Ribose	W	+	–	–
Sucrose	+	+	–	–
Trehalose	W	+	–	–
Glycerol	+	+	+	–
D-Disaccharide	–	+	ND	ND
Raffinose	+	–	ND	ND
D-Galactose	–	+	ND	ND
Antibiotics sensitivity:				
Carbenicillin (100 μg)	M	R	ND	ND
Kanamycin (30 μg)	S	R	ND	ND
Lincomycin (2 μg)	M	R	ND	S

Continued

Table 1. Continued

Characteristic	1	2	3†	4‡
Tetracycline (30 µg)	M	R	ND	R
Vancomycin (30 µg)	M	R	ND	R
GEN III tests:				
L-Histidine	+	+	−	−
L-Arginine	+	−	ND	ND
Lithium chloride	+	−	ND	ND
α-Keto-butyric Acid	+	W	ND	ND
Acetoacetic acid	+	W	+	ND
Quinone composition	Q-8	Q-8, Q-9*	Q-8	Q-8
Polar lipids	DPG, PG, PE, APGL, APL, PL, 3 GL, 2L	DPG, PG, PE, APGL, APL, PL, 2 GL, 4L	PE, 3PL, L	DPG, PG, PE, 4PL
DNA G+C content (mol%)	46.6	48.7	53.6	54.7

*Data from Huang *et al.* [1].
†Data from Wang *et al.* [5, 10].
‡Data from Srinivas *et al.* [9, 10].

species of the genus *Aliidiomarina*. Therefore, *A. taiwanensis* MCCC 1A06493^T was selected as a reference strain for further biochemical and chemotaxonomic analyses. The reference strain was obtained from the Marine Culture Collection of China (MCCC). In addition, *A. sanyensis* KCTC 32218^T and *Aliidiomarina haloalkalitolerans* MTCC 11064^T were selected as reference strains based on phylogenetic tree distances and the data were cited from their strain publications to compare the differences in various aspects within the genus.

GENOME FEATURES

The genome of strain Y-6^T was sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Beijing Genomics Institute, Beijing, PR China). The *de novo* assembly of the reads was performed using the gcType online server (http://gctype.wdcm.org/index) [20]. The quality of microbial genomes was assessed using the bioinformatic tool Check M [21]. The G+C content was calculated from the genome sequence. Rapid Annotation using Subsystem Technology (https://rast.nmpdr.org/) was used for genome sequence annotation [22]. The online tool BlastKOALA (www.kegg.jp/blastkoala/) [23] was used to reveal the metabolic pathways. Digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) values between strain Y-6^T and its closely related type strains were calculated using the Genome-to-Genome Distance Calculator (GGDC) web server (http://ggdc.dsmz.de) [24] and an ANI calculator (www.ezbiocloud.net/tools/ani) [25], respectively. The closest neighbours genome were downloaded from the NCBI databases (www.ncbi.nlm.nih.gov/genome/). Genome sequence data were uploaded to the Type (Strain) Genome Server (https://tygs.dsmz.de), for a whole genome-based taxonomic analysis [26].

The genome sequence generated 3094534 bp of clean data with 98.1% completeness and 2.4% contamination which was considered as a good reference genome for deeper analyses (≥95% completeness, ≤5% contamination) [27]. The genome sequence of strain Y-6^T consisted of 53 contigs, the N50 value was 228715 bp, the L50 value was 5 and the estimated coverage was 91%. The genome had a total of 2941 genes, including 2843 protein-coding genes, 50 tRNA and two 5S rRNA genes. The difference in general genome features between strain Y-6^T and the most closely related species are shown in Table S2 available in the online version of this article. The DNA G+C content of strain Y-6^T was 46.6mol%, distinct from *A. taiwanensis* MCCC 1A06493^T (48.7%), *A. sanyensis* KCTC 32218^T (53.6%) [5] and *A. haloalkalitolerans* MTCC 11064^T (54.7%) [9]. The dDDH values between strain Y-6^T and the reference strains of *A. taiwanensis* MCCC 1A06493^T, *A. sanyensis* KCTC 32218^T and *A. haloalkalitolerans* MTCC 11064^T were 18.3, 19.8 and 19.1%, respectively, which were below the 70% threshold value of the GGDC [27]. The ANI values between strain Y-6^T and *A. taiwanensis* MCCC 1A06493^T (74.1%), *A. sanyensis* KCTC 32218^T (68.4%) and *A. haloalkalitolerans* MTCC 11064^T (69.5%) were all lower than the 95% threshold value of the delineation of bacterial species [28]. In addition, the genome-based phylogenetic tree (Fig. S1) shows clustering of strain Y-6^T and *A. taiwanensis* MCCC 1A06493^T and definite separation from all other species. A tree was inferred with FastME 2.1.6.1 from whole-proteome-based genome BLAST distance phylogeny (GBDP) distances. The branch lengths were scaled via GBDP distance formula d_5. Branch values were GBDP pseudo-bootstrap

support values >60% from 100 replications, with an average branch support of 98.5%. The tree was midpoint-rooted. The genome sequence relatedness indicated that strain Y-6^T represents a novel species of the genus *Aliidiomarina*.

The cycling of nitrogen is very important in landfill ecosystems and has been widely researched. On the basis of the genome annotation and the BlastKOALA analysis, 22 genes related to nitrogen metabolism were found in strain Y-6^T. Based on the pathway analysis of KEGG, strain Y-6^T was found to have pathways related to cyanate hydrolysis, ammonification of nitrate and nitrite, ammonia assimilation and denitrification, which may play a role in reducing leachate pollutability, maintaining landfill system stability and increasing methane production in the landfill environment. These genes related to nitrogen metabolism were present in all the reference strains. However, compared with *A. taiwanensis* MCCC 1A06493^T, strain Y-6^T has genes related to potassium homeostasis and methyl citrate cycle, which the reference strain did not have.

PHYSIOLOGY AND CHEMOTAXONOMY

Cell morphology, size and presence of flagellum were observed by transmission electron microscopy (JEM-1230, JEOL) using cells grown on MA at 30 °C for 2 days. Cell motility was assessed using a semi-solid stabculture method with incubation at 30 °C for 2 weeks [29]. Growth conditions, including optimum growth temperature, tolerance to pH and NaCl, were determined in MB. The growth temperature test was performed at 4, 10, 20, 25, 30, 35, 37, 40, 45, 50 and 55 °C, respectively. The pH range for growth (pH 5.0–10.0, at intervals of 0.5 pH unit) was tested by using the buffer system described by Liang *et al.* [30], and the NaCl tolerance test was performed at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10 and 12% (w/v). Considering the good initial environmental growth, the pH 7.2 gradient was added in the experiment. Optimal growth was observed after 3 days of incubation, and the growth limits were determined after 14 days. Anaerobic growth for strain Y-6^T was determined with a microaerobic system (AnaeroPack-MicroAero, 2.5 l; MGC) using MA, to which 20 mM sodium thiosulphate, 5 mM sodium sulphite, 20 mM sodium sulphate, 5 mM sodium nitrite and 20 mM sodium nitrate were respectively added as potential electron acceptors [31]. Oxidase activity was tested by oxidase reagent (bioMérieux) and catalase activity was evaluated by production of oxygen bubbles in a 3.0% (v/v) aqueous hydrogen peroxide solution. Tests for H_2S production, hydrolysis of starch, tyrosine, Tweens (20, 40, 60, 80), carboxymethyl cellulose (CMC) were performed as described previously [32]. Susceptibility to antibiotics was tested on MA plates by using antibiotic discs (μg per disc unless stated otherwise; Hangzhou Microbial Reagent) of ampicillin (10 μg), carbenicillin (100 μg), ceftriaxone (30 μg), cephalothin (30 μg), chloroamphenicol (30 μg), compound sulfamethoxazale (23.75 μg/1.25 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (15 μg), lincomycin (2 μg), neomycin (30 μg), norfloxacin (10 μg), novobiocin (30 μg), oxacillin (1 μg), piperacillin (100 μg), streptomycin (10 μg), tetracycline (30 μg) and vancomycin (30 μg) after inoculation of 2 days. Biochemical characteristics were determined by API 20NE, API ZYM, API 50CH (all from bioMérieux) and GEN III MicroPlate (Biolog) systems according to the manufacturers' instructions. The reference strain *A. taiwanensis* MCCC 1A06493^T was tested simultaneously with strain Y-6^T under the same conditions.

Cells of strain Y-6^T were Gram-stain-negative, strictly aerobic, non-spore-forming rods with lateral flagella. Colonies were 0.5–1.5 mm in diameter, circular, convex, smooth and beige after growth on MA at 30 °C for 2 days. The isolate grew at 4–40 °C (optimal at 30–37 °C), pH 6.5–9.5 (optimal at pH 7.2–8.5) and in the presence of 0.5–10.0% (w/v) NaCl (optimal at 1.0–3.0%). From the results of API 20NE and API ZYM it is clear that strain Y-6^T and *A. taiwanensis* MCCC 1A06493^T were positive for catalase, oxidase, hydrolysis of tyrosine and Tweens (20, 40, 60, 80), alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, reduction of nitrates to nitrites and reduction of nitrites to nitrates. From the API 50 CH results, acids could be produced from D-glucose, D-fructose, D-mannose, sucrose, raffinose, D-toulon and D-tagatose. The results of the GEN III MicroPlate assay indicated that strain Y-6^T could utilize a diverse range of substrates, especially L-arginine, α-keto-butyric acid and acetoacetic acid, and was resistant to lithium chloride. Compared to the three reference strains, although strain Y-6^T was isolated from landfill leachate rather than a marine environment, it had a similar range of growth environments to the reference strains and was able to survive in a more acidic environment. The detailed difference in physiological and biochemical characteristics between strain Y-6^T and the most closely related species are shown in Table 1. For example, strain Y-6^T was positive for hydrolysis of gelatin (protease) while *A. taiwanensis* MCCC 1A06493^T and *A. sanyensis* KCTC 32218^T were negative. Strain Y-6^T was positive for hydrolysis of Tween 80 whereas *A. haloalkalitolerans* MTCC 11064^T was negative. *A. taiwanensis* MCCC 1A06493^T and *A. haloalkalitolerans* MTCC 11064^T were resistant to tetracycline (30 μg) and vancomycin (30 μg), conversely, strain Y-6^T was sensitive to these two antibiotics.

The fatty acid analyses of strain Y-6^T and the reference strain were based on the method described by Sasser [33]. All strains were cultured on MA at 35 °C for 2 days and harvested until the logarithmic phase, then cells were analysed using the Sherlock Microbial Identification System (MIDI; version 6.0; midi database, TSBA6) with an Agilent 6890 gas chromatograph. Respiratory quinones were determined according to the method of Minnikin *et al.* [34] and identified using HPLC-MS (Agilent 1200 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer). The polar lipids were extracted and separated by two-dimensional TLC on silica gel 60 F254 (Merck) plates (10×10 cm) according to Tindall [35]. The total lipids, aminolipids, phospholipids and glycolipids were tested after spraying with molybdophosphoric acid, ninhydrin reagent, molybdenum blue and α-naphthol/sulphuric acid ethanol (1:1) solution, respectively. The major polar lipids comprised diphosphatidylglycerol

(DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), aminoglycophospholipid (APGL), aminophospholipid (APL), phospholipid (PL), three glycolipids (GL1–3) and two unknown lipids (L1 and L2). Strain Y-6^T and *A. taiwanensis* MCCC 1A06493^T only differed in their contents of GL and L. The predominant respiratory quinone in strain Y-6^T was ubiquinone 8 (Q-8) which was in agreement with all members of the genus *Aliidiomarina* [1–11]. Compared with *A. taiwanensis* MCCC 1A06493^T, strain Y-6^T had some differences in the percentage of some fatty acids (Table S1). The major cellular fatty acids of the isolate were iso-$C_{15:0}$, $C_{16:0,}$ iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}$ *ω9c* and/or 10-methyl-$C_{16:0}$) whereas $C_{16:0}$ was not detected in *A. taiwanensis* MCCC 1A06493^T. On the basis of the results of the phenotypic, chemotaxonomic, phylogenetic and genome analyses, strain Y-6^T represents a novel species in the genus *Aliidiomarina*, for which the name *Aliidiomarina quisquiliarum* sp. nov. is proposed.

DESCRIPTION OF *ALIIDIOMARINA QUISQUILIARUM* SP. NOV.

Aliidiomarina quisquiliarum (quis.qui.li.a′rum. L. gen. fem. pl. n. *quisquiliarum* of waste, of rubbish).

Cells are Gram-stain-negative, non-spore-forming rods, motile with a lateral flagellum, strictly aerobic and approximately 0.7–1.1×1.5–2.1 μm in diameter (Fig.S2). Colonies of strain Y-6^T are 0.5–1.5 mm in diameter, circular, convex, smooth and beige after growing on MA at 35 °C for 2 days. Growth occurs at 4–40 °C (optimum, 30–37 °C), at pH 6.5–9.5 (optimum, pH 7.2–8.5) and in the presence of 0.5–10% (w/v) NaCl (optimum, 1.0–3.0%). Positive for catalase, oxidase, hydrolysis of tyrosine, gelatin (protease) and Tweens (20, 40, 60, 80), alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, trypsin, *α*-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, reduction of nitrates to nitrites and reduction of nitrites to nitrates and negative for hydrolysis of starch, CM-cellulose, valine arylamidase, cystine arylamidase, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase. Acids can be produced from D-glucose, D-fructose, D-mannose, sucrose, raffinose, D-toulon and D-tagatose. Susceptible to (μg per disc unless stated otherwise) ampicillin (10 μg), carbenicillin (100 μg), ceftriaxone (30 μg), cephalothin (30 μg), chloroamphenicol (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (15 μg), lincomycin (2 μg), neomycin (30 μg), norfloxacin (10 μg), novobiocin (30 μg), oxacillin (1 μg), piperacillin (100 μg), streptomycin (10 μg), tetracycline (30 μg) and vancomycin (30 μg), but not susceptible to compound sulfamethoxazale (23.75 μg/1.25 μg). According to the Biolog GEN III MicroPlate test, positive results for utilization of maltose, trehalose, cellobiose, sucrose, turanose, stachyose, raffinose, lactose, methyl *β*-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-*β*-D-mannosamine, *N*-acetyl neuraminic acid, *α*-D-glucose, D-mannose, D-sorbitol, D-arabitol, *myo*-inositol, glycerol, D-aspartic acid, D-serine, gelatin, L-alanine, L-arginine, L-aspartic, L-histidine, L-pyroglutamic acid, L-serine, lincomycin, glucuronamid, quinic acid, vancomycin, tetrazolium violet, tetrazolium blue, p-hydroxy-phenylacetic acid, D-lactic acid methyl ester, lithium chloride, *γ*-amino-butryric acid, *α*-hydroxy-butyric acid, *β*-hydroxy-D,L-butyric acid, *α*-keto-butyric acid, acetoacetic acid, propionic acid, acetic acid and aztreonam. The principal fatty acids are iso-$C_{15:0}$, $C_{16:0,}$ iso-$C_{17:0}$ and summed feature 9 (iso-$C_{17:1}$ *ω9c* and/or 10-methyl-$C_{16:0}$). The respiratory quinone is Q-8. The major polar lipids comprised DPG, PG, PE, APGL, APL, PL, GL1, GL2, GL3, L1 and L2 (Fig. S3).

The type strain, Y-6^T (=MCCC 1K06228^T=KCTC 82676^T), was isolated from landfill in Yiwu, Zhejiang Province, PR China. The DNA G+C content of the type strain is 46.6mol% (calculated from the genome sequence).

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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