Arenimonas malthae sp. nov., a gammaproteobacterium isolated from an oil-contaminated site

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A Gram-negative, rod-shaped bacterium (CC-JY-1^T) was isolated on nutrient agar from a soil sample collected from an oil-contaminated site located in Chyai county, Taiwan. 16S rRNA gene sequence analysis demonstrated that this isolate is unique, showing 96.7 % sequence similarity to the type strain of *Arenimonas donghaensis* and similarities of 93.0–93.8 % to species of the genera *Thermomonas*, *Lysobacter* and *Silanimonas*. The presence of ubiquinone Q-8, a polar lipid profile consisting of the major compounds diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine and the fatty acid profile were in accordance with the phylogenetic affiliation of CC-JY-1^T. DNA–DNA reassociation experiments between CC-JY-1^T and *A. donghaensis* KACC 11381^T resulted in a mean relatedness value of 32 %, indicating that strain CC-JY1^T represents a novel species in the genus *Arenimonas*, for which we propose the name *Arenimonas malthae* sp. nov. The type strain is CC-JY-1^T (=CCUG 53596^T =CIP 109310^T).

Kwon *et al.* (2007) isolated a Gram-negative organism from seashore sand for which they proposed the genus *Arenimonas*, represented by the single species *Arenimonas donghaensis*. During the characterization of micro-organisms from diesel-oil-contaminated soil samples taken near an oil refinery located in Chyai county, (Taiwan), strain CC-JY-1^T was isolated after incubation on nutrient agar at 32 °C for 3 days. Subcultivation was done on tryptone soy agar (TSA; Oxoid) at 30 °C for between 1 and 3 days.

Cell morphology of the isolate was observed under a Zeiss light microscope at ×1000 magnification using cells grown for 24 h at 30 °C on nutrient agar (Oxoid). Gram-staining was performed as described by Gerhardt *et al.* (1994). Results on the cell morphology are given in the species description.

Physiological characteristics were studied according to Kämpfer (1990) and Kämpfer *et al.* (1991). In addition, the

following test kits were used according to the instructions of the manufacturer: Biolog GN2 (Biolog), API ZYM (bioMérieux) and API 20E (bioMérieux). Antibiotic susceptibility testing was carried out using ATB STAPH 5 strips (bioMérieux) according to the manufacturer's recommendations. Presence of flexirubin-like pigments was examined by flooding the plates with 20 % (w/v) potassium hydroxide (Fautz & Reichenbach, 1980). Fluorescence was tested after plating on King's B medium (King *et al.*, 1954) after 48 h.

On nutrient agar, strain CC-JY1^T was able to grow at 15–36 °C, but not at 40 or 10 °C. The organism was able to grow on nutrient agar, TSA and R2A agar (all from Oxoid). No flexirubin-like pigments were observed. Strain CC-JY-1^T was not able to produce acid from various carbohydrates; however, carbon substrate utilization tests with organic acids as substrates showed a few positive results. In the API 20E (bioMérieux) test system, strain CC-JY-1^T was only positive for the Voges–Proskauer test (acetoin production), gelatinase and arginine dihydrolase while, in API ZYM enzyme profiling, strain CC-JY-1^T was positive for alkaline and acid phosphatases, esterase, esterase lipase, leucine arylamidase, trypsin, α-chymotrypsin and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-JY-1^T is DQ239766.

A two-dimensional thin-layer chromatogram of the polar lipids of strain CC-JY-1^T is available as supplementary material with the online version of this paper.

Table 1. Differentiating physiological characteristics of *Arenimonas* type strains

+, Positive; –, negative; (+) weakly positive. Both strains were positive in API ZYM tests for alkaline phosphatase, esterase (C4), esterase lipase (C8), trypsin, α -chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative for lipase (C14), valine arylamidase, cystine arylamidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

Characteristic	<i>A. malthae</i> CC-JY-1 ^T	<i>A. donghaensis</i> KACC 11381 ^T
API ZYM test		
Leucine arylamidase	+	–
Biochemical tests		
Acetoin production	+	–
Arginine dihydrolase	+	–
Phenylalanine deaminase	(+)	–
Nitrate reduction	+	–

naphthol-AS-BI-phosphohydrolase. More detailed results of the physiological characterization are given in the species description and in Table 1.

The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003; Young *et al.*, 2005). Analysis of the sequence data was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignments of data by CLUSTAL_X (Thompson *et al.*, 1997). A distance matrix method (distance options according to the Kimura two-parameter model), including clustering by neighbour-joining (Fig. 1), and a discrete character-based maximum-parsimony method were used (Kumar *et al.*, 2001). In each case, bootstrap values were calculated based on 1000 replications. The 16S rRNA gene sequence of strain CC-JY-1^T was 1488 bp long. Sequence similarity calculations indicated that strain CC-JY-1^T showed the highest degree of similarity to *Arenimonas donghaensis* HO3-R19^T (96.7%). Lower sequence similarities (<94%)

were found with all other genera and species shown in Fig. 1.

For quinone and polar lipid analysis, cells were grown on PYE medium (Busse *et al.*, 2005). The content of respiratory quinones was determined as described previously (Tindall, 1990; Altenburger *et al.*, 1996) but using an HPLC consisting of a JASCO PU 2080 Plus pump and JASCO UV-2075 Plus UV/Vis detector. The predominant quinone was ubiquinone Q-8, which is a characteristic trait of members of the related genera *Xanthomonas*, *Stenotrophomonas* and *Thermomonas* (Yokota *et al.*, 1992; Busse *et al.*, 2002). The polar lipid profile of CC-JY-1^T (Supplementary Fig. S1, available in IJSEM Online) consisted of the major compounds diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, moderate to minor amounts of phosphatidylmonomethylethanolamine, two unknown phospholipids, one unknown aminolipid and two unknown polar lipids and trace amounts of one unknown phospholipid and three unknown aminolipids.

The polyamines were analysed as described by Busse & Auling (1988) and Busse *et al.* (1997) but a JASCO PU 2080 Plus pump was employed. Strain CC-JY-1^T exhibited a polyamine pattern which was very similar to those of *Thermomonas* and *Xanthomonas* species (Auling *et al.*, 1991; Yang *et al.*, 1993; Busse *et al.*, 2002), consisting of the predominant compound spermidine [38.2 μ mol (g dry weight)^{–1}], minor amounts of spermine and putrescine [1.8 and 0.4 μ mol (g dry weight)^{–1}, respectively] and trace amounts of 1, 3-diaminopropane.

Fatty acid analyses were performed according to Kämpfer & Kroppenstedt (1996) for strain CC-JY-1^T and *A. donghaensis* KACC 11381^T. The results are shown in Table 2.

DNA for the determination of the G+C content was isolated by using the UltraClean microbial DNA isolation Kit (MOBIO), following the instructions supplied by the manufacturer. The G+C content of DNA was calculated as

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers given in parentheses) constructed after multiple alignments of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) were calculated and clustering with the neighbour-joining method was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at the branching points. Bar, 0.02 substitutions per nucleotide position.

Table 2. Fatty acid profiles (%) of the type strains of the genus *Arenimonas*

Fatty acid	<i>A. malthae</i> CC-JY-1 ^T	<i>A. donghaensis</i> KACC 11381 ^T
11:0 iso	2.0	3.8
11:0 iso 3-OH	5.1	7.9
13:0 iso	0.3	—
14:0 iso	0.9	1.2
14:0	0.6	1.0
15:1 iso F	3.0	2.1
15:0 iso	43.0	37.6
15:0 anteiso	0.7	—
16:0 iso	6.5	10.2
16:0	1.4	1.5
17:1 iso ω 9c	25.8	24.3
17:0 iso	8.0	8.0
17:0 anteiso	0.2	—
Summed feature 1*	0.4	1.0
Summed feature 3*	1.7	1.3

*Summed feature 1 contains 15:1 iso H and/or 13:0 3-OH. Summed feature 3 contains 16:1 ω 7c and/or 15:0 iso 2-OH.

previously published (Peña *et al.*, 2005). DNA–DNA hybridization was carried out according to the method described by Ziemke *et al.* (1998) with *A. donghaensis* KACC 11381^T, resulting in a mean DNA–DNA relatedness of 32 %.

Strain CC-JY-1^T is clearly different from *A. donghaensis*, in both phenotype (differential biochemical tests are given in Table 1) and genotype, and represents a second member of the genus *Arenimonas*, for which we propose the name *Arenimonas malthae* sp. nov.

Description of *Arenimonas malthae* sp. nov.

Arenimonas malthae (mal.tha'e. L. fem. n. *maltha* a kind of thick petroleum; L. gen. n. *malthae* of petroleum, because the type strain was isolated from an oil-contaminated site).

Cells are Gram-negative, aerobic, motile and rod-shaped. Reproduction of cells is by division and not by budding. Oxidase-, acetoin- and catalase-positive; shows an aerobic metabolism. Good growth occurs after 48 h incubation on TSA and nutrient agar at 30 °C. Colonies on complex standard media at 37 °C are transparent to brownish, circular, smooth and convex with entire edges. No flexirubin-like pigments or fluorescence are formed. Major polyamine is spermidine. Ubiquinone Q-8 is the predominant respiratory quinone. The polar lipid profile consists of the major compounds diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Moderate to minor amounts of phosphatidylmonomethyl-ethanolamine, two unknown phospholipids, one unknown aminolipid and two unknown polar lipids and trace amounts of one unknown phospholipid and three

unknown aminolipids are present. The fatty acid profile of the type strain is as follows: (percentages in parentheses): C_{11:0} iso (2.0), C_{11:0} iso 3-OH (5.1), C_{13:0} iso (0.3), C_{14:0} iso (0.9), C_{14:0} (0.6), C_{15:1} iso F (3.0), C_{15:0} iso (43.0), C_{15:0} anteiso (0.7), C_{16:0} iso (6.5), summed feature 3 (C_{16:1} ω 7c and/or C_{15:0} iso 2-OH) (1.7), C_{16:0} (1.4), iso C_{17:1} ω 9c (25.8), C_{17:0} iso (8.0) and C_{17:0} anteiso (0.2). The type strain utilizes fumarate, propionate, glutarate, pyruvate, L-alanine, L-aspartate, L-leucine, L-proline, L-serine and DL-3-hydroxybutyrate, utilizes L-phenylalanine weakly and is unable to utilize acetate, *cis*-aconitate, L-malate and mesaconate after 7 days of incubation. The following carbon sources are oxidized (positive with the Biolog GN2 system): glycogen, pyruvic acid methyl ester, β -hydroxybutyric acid, L-alaninamide, L-alanine, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-proline, L-serine, α -ketovaleric acid (weakly), succinic acid (weakly), L-phenylalanine (weakly) and L-asparagine (weakly). The following substrates are not utilized as carbon sources: dextrin, α -cyclodextrin, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, adonitol, L-arabitol, D-cellobiose, i-erythritol, D-fructose, L-fructose, D-galactose, gentiobiose, α -D-glucose, *myo*-inositol, α -D-lactose, lactulose, maltose, D-mannitol, D-melibiose, methyl β -D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, glucuronamide, D-alanine, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-pyrogutamic acid, D-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, α -D-glucose 6-phosphate and D-glucose 6-phosphate. Aesculin is not hydrolysed. Positive tests (API ZYM) are observed for arginine dihydrolase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, trypsin, α -chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, with negative results for lipase, valine arylamidase, cystine arylamidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. The type strain is sensitive to gentamicin, erythromycin, tetracycline, minocycline, rifampicin, norfloxacin and levofloxacin, while it is resistant to penicillin, cotrimoxazole, plindamycin, vancomycin, teicoplanin, quinupristin-dalfopristin, coag(-)oxacillin and oxacillin. The G + C content of the type strain is 70.4 mol%.

The type strain is CC-JY-1^T (=CCUG 53596^T =CIP 109310^T), isolated from a diesel-oil-contaminated soil sample.

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