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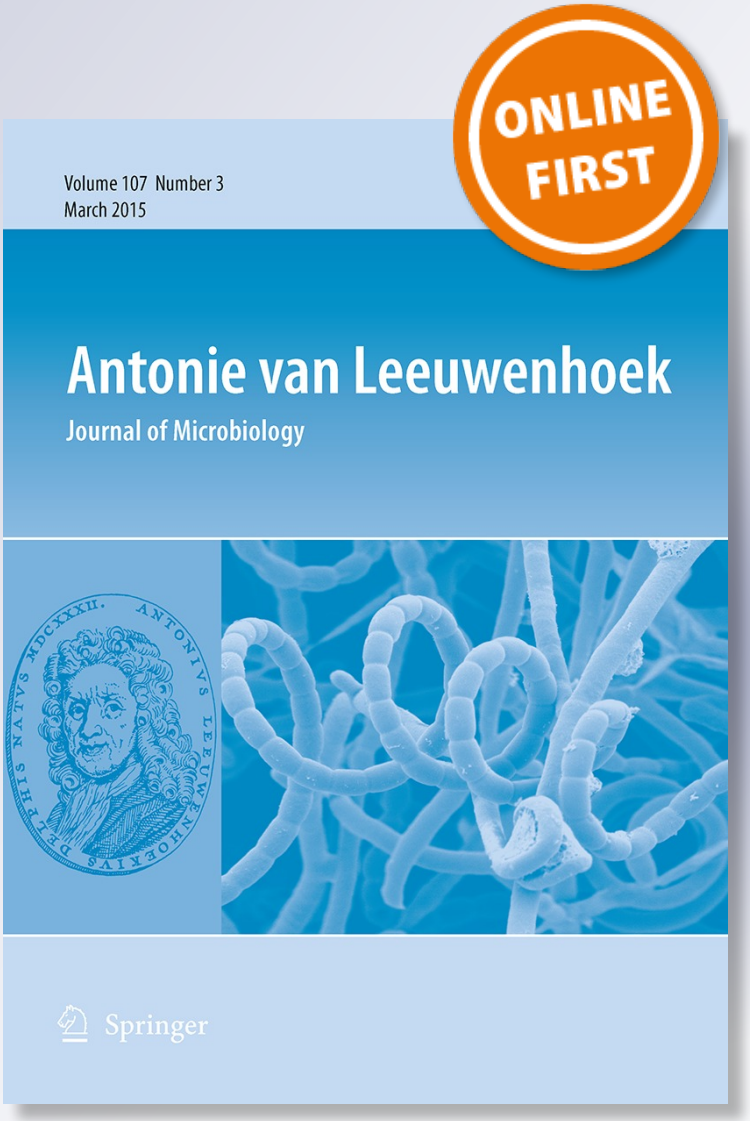

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ORIGINAL PAPER

Arenimonas taoyuanensis sp. nov., a novel bacterium isolated from rice-field soil in China

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Abstract A Gram-stain negative, aerobic, rod-shaped bacterial strain, YN2-31A^T, was isolated from rice-field soil, Taoyuan Village, Yunnan province of China. The bacterium was observed to grow at 20–45 °C (optimum 28 °C), at pH 5.0–10.0 (optimum 7.0), and in the presence of 0–2 % (w/v) NaCl (optimum 0–1 %). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YN2-31A^T is most closely related to *Arenimonas daejeonensis* DSM 18060^T (96.1 %), *Arenimonas malthae* DSM 21305^T (95.9 %), *Arenimonas donghaensis* DSM 18148^T (95.1 %), *Arenimonas composti* DSM 18010^T (94.8 %) and *Arenimonas maotaiensis* JCM 19710^T (94.8 %). The major cellular fatty acids (>10 %) were found to be iso-$C_{18:1}$ $\omega 9c$, iso-$C_{15:0}$, Sum In Feature 3 ($C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$), and $C_{16:0}$. The major ubiquinone was identified as Q-8 and the major cellular polar lipids were identified as diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and unidentified phospholipids. The genomic DNA G+C content was determined to be 72.3 mol%. The results of the phylogenetic, genetic, phenotypic and chemotaxonomic analyses suggest that strain YN2-31A^T represents a novel species of the genus *Arenimonas*, for which the name *Arenimonas taoyuanensis* sp. nov. is proposed. The type strain is YN2-31A^T (=DSM 26777^T = CCTCC AB2012964^T).

Keywords *Arenimonas taoyuanensis* · Novel species · Rice · Rhizosophere

Shi-Ying Zhang and Wei Xiao have contributed equally to this work.

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Introduction

Since paddy fields regularly experience alternation of drying-wetting, anaerobic and methane-producing bacteria are reported to be common inhabitants of paddy soils (Akasaka et al. 2003; Dianou et al. 2001; Ueki et al. 2006). Nevertheless, both aerobic (Lim et al. 2007; Aslam et al. 2009; Zhang et al. 2011) and facultatively anaerobic bacteria (Lakshmi et al. 2009) have been also isolated. Due to its natural environment conditions, Taoyuan Village, Yongsheng County, Yunnan province of China is a prominent eco-site known for the highest rice yield. (Katsura et al. 2008; Li et al. 2009; Welch et al. 2010). During an investigation of microbial diversity of

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paddy soil associated with the roots of *Oryzae sativa* growing in Taoyuan village, a Gram-stain negative, aerobic, rod-shaped bacterium YN2-31A^T was isolated. The phylogenetic analysis based on 16S rRNA gene sequences revealed that this bacterium is closely related to the members of the genus *Arenimonas*.

The genus *Arenimonas,* family *Xanthomonadaceae* of the class *Gammaproteobacteria,* was first proposed by Kwon et al. (2007) to accommodate *Arenimonas donghaensis* HO3-R19^T (the type species of the genus). Bacteria of this genus are aerobic, Gram-negative, non-spore-forming and rod-shape with the major isoprenoid quinone Q-8. At the time of writing, there are eight species were described in the genus *Arenimonas*, including *A. donghaensis* (Kwon et al. 2007), *A. malthae* (Young et al. 2007), *A. composti* (Jin et al. 2007; Aslam et al. 2009), *A. oryziterrae* (Aslam et al. 2009), *A. metalli* (Chen et al. 2012), *A. daejeonensis* (Jin et al. 2012), *A. daechungensis* (Huy et al. 2013) and *A. maotaiensis* (Yuan et al. 2014). A misclassified species "*Aspromonas composti"* TR7-09^T (Jin et al. 2007) was transfered to the genus *Arenimonas* as *A. composti* in 2009 (Aslam et al. 2009). In this study, we show that strain YN2-31A^T represent a novel species of the genus *Arenimonas*, for which the name *Arenimonas taoyuanensis* sp. nov. is proposed.

Materials and methods

Isolation and cultivation of strain

During the characterization of bacteria from paddy soil samples collected from super-high yield rice field located in Taoyuan Village, Yongsheng County, Yunnan province, China (N26°13′33″, E100°33′29″), strain YN2-31A^T was isolated as described in Lim et al. (2007) using R2A at 28 °C. The isolate is maintained on R2A medium slants and preserved at −80 °C as suspension in R2A containing 20 % (v/v) glycerol. The type strains, *A. malthae* DSM 21305^T, *A. donghaensis* DSM 18148^T and *A. composti* DSM 18010^T were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ) and used as reference strains throughout this work.

Determination of 16S rRNA gene sequences and phylogenetic analyses

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were carried out as described elsewhere (Cui et al. 2001). For the determination of almost full-length of 16S rRNA gene sequences, the PCR amplicons were ligated into the pMD19-T vector using a pMD19-T cloning kit (TaKaRa) according to the manufacturer's instructions. The inserted 16S rRNA genes were sequenced with the M13 primers of the cloning kit. The resulting 16S rRNA gene sequences of strain YN2-31A^T was checked for chimeras using the software Mallard (version 1.02) (Ashelford et al. 2006).

Sequence similarities between the strain and closely related taxa were evaluated using the Nucleotide Similarity Search Program (http://eztaxon-e.ezbiocloud.net/; Kim et al. 2012) and aligned using the program CLUSTAL_X (Thompson et al. 1997). Phylogenetic trees using the neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) algorithms were constructed by the MEGA 5.0 software (Tamura et al. 2011). Evolutionary distances were calculated according to the algorithm of the Kimura's two-parameter model (Kimura 1980) for the NJ method. Bootstrap analysis was used to evaluate tree topology by means of 1000 resamplings (Felsenstein 1985). The 16S rRNA gene sequence of the strain *Escherichia coli* ATCC 11775^T (X80725) was used as the outgroup.

Phenotypic and physiological tests

Gram staining was performed as described in Smibert and Krieg (1994). Cell morphology and flagellum type was observed by using transmission electron microscopy (JEM-2100). Motility was observed via the hanging-drop method (Suzuki et al. 2001). Growth at various temperatures (4, 10, 15, 20, 25, 28, 37 and 45 °C) was determined on R2A agar. The pH range for growth was investigated between pH 5.0 and 10.0 (in increments of 1 pH unit) on R2A broth with the buffer system described by Xu et al. (2005). To test NaCl tolerance, growth at various NaCl concentrations (0, 1, 2, 3, 4, and 5 %, w/v) was investigated in R2A agar. Growth under anaerobic conditions was determined after incubation in an anaerobic jar (GasPak Anaerobic systems; BBL) on R2A agar. Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovacs' reagent (Kovacs 1956), respectively. Degradation of cellulose, gelatin, starch, chitin and Tweens 20, 40 and 80 was determined according to the protocols described by Cowan and Steel (1965). Tests of carbon utilization, tests for acid production

from carbohydrates and tests for other enzyme activities were assayed by using the API 20 NE, API 50 CH and API ZYM (bioMérieux) according to the manufacturer's instructions. Antibiotic resistance was determined by the disc diffusion method using commercial antibiotic-impregnated discs on R2A agar (Bauer et al. 1966). The results were estimated according to the inhibition zone.

Chemotaxonomic analysis

Chemotaxonomic analyses were performed using strain YN2-31A^T and the reference strains *A. malthae* DSM 21305^T, *A. donghaensis* DSM 18148^T and *A. composti* DSM 18010^T. Isoprenoid quinones were extracted according to the method of Collins et al. (1977) and analysed by HPLC as described by Tamaoka et al. (1983). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin et al. 1977). Analysis of the cellular fatty acid pattern was carried out using the method described by Sasser (1990) with Sherlock Microbial Identification System version 6.0 (MIDI system) and the standard MIS Library TSBA6. Biomass for isoprenoid quinones, polar lipids and fatty acid analysis of all strains were harvested at exponential growth phase after 4 days at 28 °C from R2A plates.

The genomic DNA G+C content was determined by HPLC according to Mesbah et al. (1989); DNA was extracted according to the method of Cui et al. (2001).

Results and discussion

An almost complete 16S rRNA gene sequence of strain YN2-31A^T (1499 bp; GenBank accession number KC237721) was obtained. Comparison of the sequences with those available from public databases revealed that the newly isolated bacterium is closely related to the bacteria of the genus *Arenimonas*. In the NJ phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences, strain YN2-31A^T was placed in a cluster with other species of the genus *Arenimonas* and was found to be closely related to *A. daejeonensis* DSM 18060^T (96.1 %), *A. malthae* DSM 21305^T (95.9 %), *A. donghaensis* DSM 18148^T (95.1 %), *A. composti* DSM 18010^T (94.8 %) and *A. maotaiensis* JCM 19710^T (94.8 %), which are lower values than the borderline used for definition of bacterial species (i.e. 97 %) as proposed by Stackebrandt & Goebel (1994). The phylogenetic analyses using the ML and MP algorithms confirmed the distinct phylogenetic position of strain YN2-31A^T (Supplementary Fig. S1).

Strain YN2-31A^T was observed to be Gram-stain negative, aerobic, rod-shaped with 1–5 lateral thin flagella (Supplementary Fig S2). Optimal growth conditions were found to be 28 °C, pH 7 on R2A medium, and besides that growth was found to occur at 20–45 °C, pH 5–10 and in the presence of 0–2 % (w/v) NaCl. Physiological and biochemical characteristics of strain YN2-31A^T are presented in Table 1 and S1 and in the new species description. Strain YN2-31A^T was found to be susceptible to amikacin (30 mg), chloramphenicol (30 mg), ciprofloxacin (5 mg), erythromycin (15 mg), gentamicin (10 mg), norfloxacin (10 mg), rifampicin(5 mg), tobramycin (10 mg), clindamycin (2 mg), novobiocin (5 mg), and tetracycline (30 mg). Resistant to ampicillin (10 mg), penicillin G (10 IU) and vancomycin (30 mg).

The isoprenoid quinone of strain YN2-31A^T, ubiquinone-8 (Q-8), was also reported as typical quinone for *Arenimonas* species (Jin et al. 2007; Kwon et al. 2007; Young et al. 2007). The major cellular fatty acids (>10 %) of strain YN2-31A^T were identified as iso-$C_{18:1}$ $\omega 9c$, iso-$C_{15:0}$, Sum In Feature 3 ($C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$), and $C_{16:0}$. The cellular fatty acid profiles of strain YN2-31A^T were similar to those of other closely related members of the genus *Arenimonas*. For example, iso-$C_{15:0}$ was found to be the major fatty acid in all studied *Arenimonas* species and YN2-31A^T. But minor differences in the proportions of some fatty acids could be observed such as iso-$C_{16:0}$ and iso-$C_{17:1}$ ω9c.

The polar lipid profile of YN2-31A^T was found to consist of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the major components, five unidentified phospholipids, one unidentified aminolipid and trace amounts of two unidentified polar lipids are found in moderate to minor amounts (Supplementary Fig S3). Phosphatidylethanolamine, phosphatidylglycerol, and unidentified phospholipids are present in all *Arenimonas* species studied here, although in different proportions depending upon the species analyzed.

In conclusion, the phenotypic and chemotaxonomic properties and phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YN2-31A^T is a member of the genus *Arenimonas* and represents a genomic species that is separate from validly named

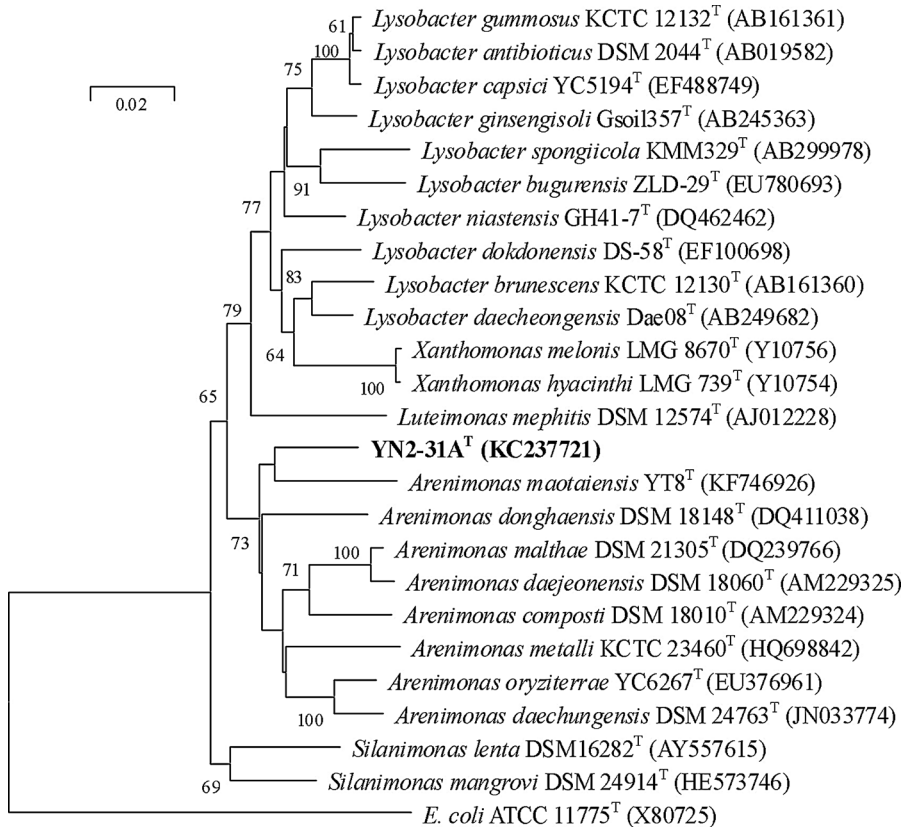

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain YN2-31A^T and other species of the genus *Arenimonas*. Bootstrap values (expressed as percentages of 1000 replications) of above 50 % are shown at *branch points*. *Bar* 0.02 substitutions per nucleotide position

Arenimonas species. The name *Arenimonas taoyuanensis* sp. nov. for strain YN2-31A^T is proposed.

Description of *Arenimonas taoyuanensis* sp. nov.

Arenimonas taoyuanensis (tao.yuan.en'sis N.L. fem. adj. *taoyuanensis*, pertaining to Taoyuan Village, a special eco-site which keeps the highest yield records of rice cultivation in small planting areas in Yunnan province, China, where the type strain was isolated).

Cells are Gram-stain negative, aerobic, motile and rod-shaped (0.3–0.4 × 2.2–3.0 μm) with 1–5 lateral thin flagella. Good growth is observed on R2A agar, nutrient agar and tryptic soy agar. Colonies are yellowish white, circular, convex and opaque with regular margins. Growth occurs at NaCl concentrations of 0–2 % (w/v) (optimum 0–1 %), 20–45 °C (optimum 28 °C) and pH 5–10 (optimum pH 7). Oxidase-positive but catalase-negative. Hydrolyses Tween 20 and gelatin but does not hydrolyse chitin, starch, cellulose, Tween 40 and Tween 80. Cells are positive for hydrolysis of aesculin ferric citrate and gelatin, but negative for 4-nitrophenyl-β-D-galactopyranoside, nitrate or nitrite reduction, indole production, arginine dihydrolase, glucose fermentation, urease, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-gucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate or phenylacetic acid (in API 20NE). Positive results for the following enzyme activities (API ZYM test strip): alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, but negative results for the following enzyme activities: lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. According to API 50CH tests, acid is produced from esculin, but not from glycerol, L-arabinose, D-ribose, D-xylose, D-fructose, D-mannose, L-rhamnose, D-cellobiose, glycogen, D-saccharose, D-trehalose, D-mannitol, starch, D-glucose, D-lactose,

Table 1 Physiological and biochemical characteristics that differentiate strain YN2-31A^T from its closely related species of the genus *Arenimonas*

Characteristic	*A. taoyuanensis* YN2-31A^T	*A. composti* DSM 18010^T	*A. malthae* DSM 21305^T	*A.donghaensis* DSM 18148^T	*A. daejeonensis* DSM 18060^T [b]	*A. maotaiensis* JCM 19710^T [c]
Source	Rice-field soil	River sediment	Oil-contaminated soil	Oil-contaminated seashore sand	Compost	Fresh water
Colony colour	Yellowish white	Creamy white	Brownish	Yellowish white	Creamy white to yellowish	Translucent white
NaCl range for growth (%, w/v)	0–2	0–2	0–4	0–3	0–3	0–0.5
Catalase	−	−	+	+	+	+
API ZYM						
Leucine arylamidase	+	−	+	+	+	+
Valine arylamidase	+	−	−	−	−	+
Cystine arylamidase	+	−	+	−	−	−
Trypsin	+	−	+	+	+	+
Acid phosphatase	+	+	−	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	−	−	−	+	+
DNA G+C content (mol%)[a]	72.3	70.8	70.4	65.0	68.3	66.6
Major polar lipids	PG, DPG, PE, PL	PME, PE, PG, PL	DPG, PG, PE, PL, APL AL, L	DPG, PE, PG, PL, APL	DPG, PE, PG, PME, PL, APL	PE, PL, AL, L

+ positive or present, − negative or absent

[a] Data of genomic DNA G+C content for strain *Arenimonas malthae* DSM 21305^T from Young et al. (2007), *Arenimonas composti* DSM 18010^T from Jin et al. (2007) and *Arenimonas donghaensis* DSM 18148^T from Kwon et al. (2007)

[b] Data taken from Jin et al. (2012)

[c] Data taken from Yuan et al. (2014)

erythritol, D-arabinose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside D-galactose, L-sorbose, dulcitol, methyl-α-D-glucopyranoside, inositol, D-sorbitol, salicin, inulin, methyl-α-D-mannopyranoside, *N*-acetyl-glucosamine, amygdaline, arbutine, D-maltose, D-melibiose, D-melezitose, D-raffinose, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The major cellular fatty acids (>10 %) are iso-$C_{18:1}$ $\omega 9c$, iso-$C_{15:0}$, Sum In Feature 3 ($C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$), and iso-$C_{16:0}$. The major respiratory quinone is ubiquinone Q-8. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine and unknown phospholipids. The genomic DNA G+C content of the type strain YN2-31A^T is 72.3 mol%.

The type strain, YN2-31A^T (=DSM 26777^T -= CCTCC AB2012964^T), was isolated from a field of rice (*Oryza sativa L.*)

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Conflict of interest The authors declare that they have no conflict of interest.

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