Arenimonas oryzae sp. nov., isolated from a field of rice (*Oryza sativa* L.) managed under a no-tillage regime, and reclassification of *Aspromonas composti* as *Arenimonas composti* comb. nov.

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The taxonomic position of a novel bacterial strain, YC6267^T isolated from a field of rice (*Oryza sativa* L.) managed under a no-tillage regime in Jinju, Korea, was studied using a polyphasic taxonomic approach. Cells of the strain were Gram-stain-negative, rod-shaped and aerobic. It grew at 15–37 °C (optimum at 28 °C). Growth of the strain occurred between pH 5.0 and 10.0, with an optimum of pH 7.0–8.0. The G+C content of the total DNA was 65.8 mol%. The 16S rRNA gene sequence of the strain was most closely related to species of the genera *Arenimonas* (95.6–94.4 %) and *Aspromonas* (95.1 %), with <95.0 % similarity to species of the genus *Lysobacter* and other genera of the family *Xanthomonadaceae*. Chemotaxonomic data (major quinone Q-8; major polar lipids phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol; and major fatty acids iso-C_{15:0}, iso-C_{14:0}, iso-C_{16:0}, and iso-C_{17:1ω9c}) supported the affiliation of strain YC6267^T to the genus *Arenimonas*. Phylogenetic analysis based on 16S rRNA gene sequences and biochemical and physiological characteristics strongly supported the genotypic and phenotypic differentiation of strain YC6267^T from described species of the genus *Arenimonas*. Strain YC6267^T, therefore, represents a novel species, for which the name *Arenimonas oryzae* sp. nov. is proposed. The type strain is YC6267^T (=KCTC 22247^T =DSM 21050^T). In addition, the reclassification of *Aspromonas composti* as *Arenimonas composti* comb. nov. is proposed (type strain TR7-09^T =KCTC 12666^T =DSM 18010^T). A common line of descent and a number of shared phenotypic traits support this reclassification.

The genus *Arenimonas*, grouped in the family *Xanthomonadaceae*, belonging to the *Gammaproteobacteria*, was proposed by Kwon *et al.* (2007). At present, the genus has only two species with validly published names, *Arenimonas donghaensis* (Kwon *et al.*, 2007) and *Arenimonas malthae* (Young *et al.*, 2007). The genus *Arenimonas* has the following characteristics: cells are aerobic, Gram-negative, non-spore-forming rods that are oxidase- and catalase-positive. Nitrate and nitrite are not reduced. Major isoprenoid quinone is Q-8. Predominant cellular fatty acids are iso-branched, such as iso-C_{16:0}, iso-C_{15:0} and iso-C_{17:1ω9c} (Kwon *et al.*, 2007).

The bacterial communities of two rice fields, managed under conventional and no-tillage (zero-tillage) regimes,

were investigated using an improved culture method. This method was based on a combination of long incubation, sonication, selection of microcolonies with the help of a magnifying lens, increasing the number of inoculation plates of each dilution, half-strength R2A agar, 25 % soil extract (v/v) and some additional modifications (Hamaki *et al.*, 2005; Janssen *et al.*, 2002). The half-strength R2A agar consisted of 0.25 g yeast extract, 0.25 g proteose peptone no. 3, 0.25 g Casamino acids, 0.25 g glucose, 0.25 g soluble starch, 0.15 g sodium pyruvate, 0.15 g K₂HPO₄, 0.03 g MgSO₄ and 15 g agar in 1 litre distilled water. Many taxonomically unknown bacterial strains were successfully isolated.

A total of four soil samples were collected from the rhizosphere of each field at the zero phase (pre-sowing), vegetative, reproductive and ripening phases of rice, at Gyeongsang National University farm (Daegok valley) located in the north-east of Jinju, Korea during 2007.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YC6267^T is EU376961.

Three supplementary figures and one supplementary table are available with the online version of this paper.

Samples were collected at a depth of 15–20 cm and a width of 6–10 cm from five randomly selected points in each field after removing the upper 3–5 cm of soil, straw and debris. After sonication and serial dilution in 50 mM phosphate buffer (pH 7.0), the samples were spread on modified half-strength R2A agar plates. Plates were incubated at 28 °C for more than 1 month. Single colonies were purified by transferring them onto new half-strength R2A agar plates, and these were incubated again. One novel bacterial strain, YC6267^T, was selected from the many strains isolated from samples collected from the rice field under the no-tillage regime at the zero growth phase. The novel strain was identified on the basis of 16S rRNA gene sequence and other chemotaxonomic and genotypic characteristics, as determined for *Lysobacter capsici* (Park *et al.*, 2008). It was routinely cultured on half-strength R2A agar at 28 °C and maintained in a suspension of glycerol (15%, w/v) and half-strength R2A broth at –70 °C.

Cell morphology was observed under a Nikon light microscope at ×1000 and a transmission electron microscope (Hitachi, model H-600), with cells grown for 1 day at 28 °C in half-strength R2A broth. Catalase and oxidase tests were performed using the procedures outlined by Cappuccino & Sherman (2002). The physiological properties of strain YC6267^T were determined using previously described tests: hydrolysis of casein, aesculin, gelatin, starch and urea (Brown, 2007), hippurate (Kinyon & Harris, 1979), elastin (Ohman *et al.*, 1980), guanine and adenine (Wallace *et al.*, 1995), cellulose and xylan (Ten *et al.*, 2004) and Tween 80 (Atlas, 1993). Enzyme activities and acid production from different carbohydrates were determined by using API ZYM, ID 32E and API 20E kits, at 30 °C according to the instructions of the manufacturer (bioMérieux). API ZYM strips were read after 5 h incubation. The assimilation of single carbon substrates was determined by using Biolog GN2 and API 20 NE kits at 30 °C after 48 h incubation. Growth at different temperatures (4, 15, 20, 28, 37 and 42 °C) was tested on half-strength R2A agar. Anaerobic growth was tested at 28 °C by pouring a thick layer of vaspar (50% petrolatum, 50% paraffin) onto the surface of inoculated half-strength R2A broth in 35 ml screw-capped glass tubes (Costilow, 1981). Growth at different temperatures and pH was assessed after 3 days of incubation. Salt tolerance was tested in half-strength R2A broth supplemented with 0–10% (w/v) NaCl after 7 days of incubation. Duplicate antibiotic-sensitivity tests were performed using filter-paper discs containing the following: tetracycline, kanamycin, ampicillin (Sigma), streptomycin and rifampicin, each at 10, 50 or 100 µg ml⁻¹. Discs were placed on half-strength R2A plates spread with a suspension of strain YC6267^T and the plates were incubated at 28 °C for 3 days.

To determine 16S rRNA gene sequences, genomic DNA was extracted using a commercial genomic DNA extraction kit (Core Biosystem). The gene was amplified by PCR from a small amount (1.5 ng) of purified genomic DNA by using primers 27F and 1492R, and the purified PCR product was

sequenced by GenoTech Inc. (Daejeon, Korea) (Lane, 1991). The 16S rRNA gene sequences were compiled using SeqMan software (DNASTAR) and the sequences of related taxa were obtained from the GenBank database. Identification of phylogenetic neighbours was initially carried out by using BLAST (Altschul *et al.*, 1997) and FASTA (Pearson & Lipman, 1988) programs against the database of type strains with validly published prokaryotic names (<http://www.bacterio.cict.fr/>). The 50 sequences with the highest scores were then selected for calculation of pairwise sequence similarity using a global alignment algorithm, which was implemented at the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Multiple alignments were performed for 35 closely related taxa including *Escherichia coli* ATCC 11775^T (used as an outgroup) by using the CLUSTAL_X program (Thompson *et al.*, 1997). Gaps were edited using the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using a neighbour-joining method (Saitou & Nei, 1987) in the MEGA4 program (Tamura *et al.*, 2007), and by maximum-parsimony (Fitch, 1971) and maximum-likelihood in PHYLIP software, version 3.6 (Felsenstein, 2002), with bootstrap values based on 1000 replications (Felsenstein, 1985).

For the measurement of G + C content of chromosomal DNA, genomic DNA of strain YC6267^T was extracted and purified as described by Ausubel *et al.* (1995). It was then enzymically degraded into nucleosides, and G + C content was determined as described by Mesbah *et al.* (1989) using reverse-phase HPLC. Cellular fatty acids were analysed using strain YC6267^T and reference strains grown on R2A agar for 2 days at 28 °C. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System version 4.0 software (MIDI). Fatty acids analysed by GC (Hewlett Packard 6890) were identified using the Microbial Identification software package (Microbial ID). The quinone system and polar lipids were determined by HPLC and two-dimensional TLC, respectively, as described by Tindall (1990) and Lechevalier *et al.* (1977).

The novel strain was Gram-stain negative and non-motile. Cells were short rods (0.4–0.5 × 1.2–2.1 µm) (Supplementary Fig. S1, available in IJSEM Online) and occurred singly, in pairs and/or in clusters. Colonies grown on half-strength R2A agar plates for 3 days were smooth, circular, yellowish to creamy-white in colour and 1–2 mm in diameter. Fruiting bodies were not observed. Growth was aerobic. The strain grew well on full-strength R2A agar, nutrient agar, 1.0% trypticase soy broth (w/v) plus 1.5% agar, and EMB agar, but did not grow on MacConkey agar, full-strength trypticase soy agar (TSA) or LB agar. All culture media were from Difco. Physiological characteristics and enzymic activities of strain YC6267^T are summarized in the species description, and comparison of selective characteristics with related type strains is given in Table 1.

Table 1. Differential phenotypic characteristics between strain YC6267^T and closely related taxa

Strains: 1, *Arenimonas oryzae* sp. nov. YC6267^T; 2, *Arenimonas malthae* CIP 109310^T; 3, *Arenimonas donghaensis* DSM 18148^T; 4, *Aspromonas composti* KCTC 12666^T; 5, *Lysobacter koreensis* KCTC 12204^T. All data from this study unless indicated. All strains were positive for alkaline phosphatase, esterase (C-4), esterase lipase (C-8), naphthol-AS-BI-phosphohydrolase and hydrolysis of casein, and negative for α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase. +, Positive; –, negative; NA, not available. Indole production and hydrolysis of starch and xylan were negative in all strains.

Characteristic	1	2	3	4	5
Cell size (μ m)	0.5–0.6 $\times$ 2.3–2.6	0.3–0.4 $\times$ 1.6–2.7	0.4–0.6 $\times$ 1.3–3.0 ^{a*}	0.4–0.5 $\times$ 1.0–1.8 ^b	0.5–0.8 $\times$ 1.5–2.0 ^c
Motility	–	+	+ ^a	+ ^b	– ^c
Source of isolation	Rice rhizosphere	Oil-contaminated soil ^d	Seashore sand	Compost and river-sediment ^b	Soil ^c
Temperature optimum ($^{\circ}$ C)	28	30	28	30	30
Colony colour	Yellowish to creamy-white	Transparent to brownish	Yellowish-white	Creamy-white	Yellow
Nitrate reduction	–	+	–	–	–
NaCl (% w/v) test					
Optimum	0	0	1.0–2.0	0	0
Range	0	0–2.0	0–3.0	0–2.0	0–2.0
No growth	1.0	3.0	4.0	3.0	3.0
Catalase	+	+	+	–	+
Oxidase	+	+	+	+	–
Hydrolysis of:					
Aesculin	–	–	–	–	+
Arginine	–	+	–	+	–
Cellulose	+	–	–	–	–
Elastin	+	–	+	–	+
Gelatin	+	+	+	–	+
Hippurate	–	+	+	+	–
Phenylalanine	–	+	–	–	–
Urea	–	–	–	+	–
Acetoin production	–	+	–	–	–
API ZYM tests					
α -Chymotrypsin	+	+	+	+	–
Acid phosphatase	+	+	+	+	–
Cystine arylamidase	+	–	–	–	–
Leucine arylamidase	+	+	–	+	+
Lipase (C-14)	+	–	–	–	–
Trypsin	+	+	+	–	–
Valine arylamidase	+	–	–	–	–
Major polar lipids [†]	DPG, PE, PG	DPG, PE, PG ^d	DPG, PE, PG, PL, AL ^a	PE, PG, PME ^b	NA
DNA G + C content (mol%)	65.8	70.4 ^d	65.0 ^a	70.8 ^b	68.9 ^c

*Data taken from: ^a, Kwon *et al.*, 2007; ^b, Jin *et al.*, 2007; ^c, Lee *et al.*, 2006; ^d, Young *et al.*, 2007.

[†]AL, unknown amino group-containing lipids; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid; PME, phosphatidylmethylethanolamine.

The cellular fatty acid profiles of strain YC6267^T and related *Arenimonas* type strains are shown in Supplementary Table S1. The major (>5.0%) cellular fatty acids of strain YC6267^T included iso-C_{15:0} (30.6%), iso-C_{17:1} ω 9c (16.1%), iso-C_{14:0} (13.2%), iso-C_{16:0} (10.2%), iso-C_{11:0} 3-OH (7.1%) and iso-C_{15:1} F (6.2%). The major fatty acids of the novel strain were similar to those of species of the genus *Arenimonas*; however, some differences were found. C_{16:1} ω 11c and C_{16:1} ω 7c alcohol were detected only in strain YC6267^T.

Similarities in the pattern of major fatty acids between strain YC6267^T, *Aspromonas composti* KCTC 12666^T and species of the genus *Arenimonas* indicated that they might belong to the same genus. However, they could be easily differentiated from closely related strains on the basis of different amounts of the major fatty acids and the presence of some minor components (Supplementary Table S1). The major fatty acid profile of *Aspromonas composti* KCTC 12666^T determined on R2A agar (this study) was similar to that on TSA (Jin *et al.* 2007), except for the presence of iso-

C_{15:0} 2-OH, C_{12:1} AT 11–12 and C_{10:0}, which appeared in minor amounts. *Lysobacter koreensis*, which has 94.8 % 16S rRNA gene sequence similarity to strain YC6267^T, showed a very different fatty acid profile. The following polar lipids were present: phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol; and some unknown ALs (amino group-containing lipids) (Supplementary Fig. S2), which suggested the affiliation of strain YC6267^T to the genus *Arenimonas* (Kwon *et al.*, 2007).

The DNA G + C content of strain YC6267^T was 65.8 mol%. The predominant quinone was ubiquinone 8 (Q-8), as with closely related taxa. The partial 16S rRNA gene sequence of strain YC6267^T was 1430 bp. Sequence similarity calculations obtained by pairwise comparisons indicated that the closest relatives of novel strain YC6267^T were *Arenimonas malthae* CC-JY-1^T (95.6 %), *Aspromonas composti* TR7-09^T

(95.1 %) and *Arenimonas donghaensis* HO3-R19^T (94.4 %), and lower similarities (<95.0 %) were found with the other genera of the family *Xanthomonadaceae*. Phylogenetic trees based on the neighbour-joining (Fig. 1), maximum-likelihood (Supplementary Fig. S3) and maximum-parsimony methods (data not shown) showed clearly that strain YC6267^T represented a distinct lineage close to the genus *Arenimonas*, supported by a high bootstrap value. Strain TR7-09^T, which was previously classified as *Aspromonas composti*, also formed a distinct lineage close to the genus *Arenimonas* (Fig. 1 and Supplementary Fig. S3). Low levels (<97.0 %) of 16S rRNA gene sequence similarity between strain YC6267^T and closely related type strains of the genus *Arenimonas* eliminated the need for the DNA–DNA hybridization test (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). The phylogenetic analysis (Fig. 1 and

Fig. 1. Phylogenetic tree reconstructed from the comparative analysis of 16S rRNA gene sequences showing the relationships of strain YC6267^T to closely related taxa. This phylogenetic tree was reconstructed by using the neighbour-joining method and Jukes & Cantor evolutionary distance matrix data obtained from aligned nucleotides. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 1 substitution per 100 nucleotide positions.

Supplementary Fig. S3), G + C content, other physiological and biochemical characteristics (Table 1) and fatty acid analysis profile (Supplementary Table S1) clearly distinguish strain YC6267^T from all species of the genus *Arenimonas*, as well as from *Aspromonas composti*. Thus, on the basis of the facts given above, we propose that strain YC6267^T represents a novel species of the genus *Arenimonas*, for which the name *Arenimonas oryzae* sp. nov. is suggested. We also propose the reclassification of *Aspromonas composti*, as *Arenimonas composti* comb. nov. *Aspromonas composti* was reclassified on the basis of phylogenetic analysis (Fig. 1) and shared phenotypic characteristics: Gram-stain-negative, non-spore-forming rods, oxidase positive, no reduction of nitrate and nitrite, major isoprenoid quinone Q-8. Other common and distinguishing characteristics are shown in Table 1 and Supplementary Table S1.

Description of *Arenimonas oryzae* sp. nov.

Arenimonas oryzae (o.ry.za.ter'rae. L. n. *oryza* rice; L. n. *terra* earth, soil; N.L. gen. n. *oryzae* of the rice soil or field, referring to the source of isolation of the type strain).

Cells are Gram-stain-negative, aerobic, non-spore-forming, non-motile, short rods, 0.5–0.6 µm wide by 2.3–2.6 µm long. Occur singly, in pairs and/or in clusters. Colonies grown on half-strength R2A at 28 °C for 3 days are 1–2 mm in diameter, yellowish to creamy-white in colour, smooth and circular in shape. The temperature range for growth is 15–37 °C with an optimum at 28 °C. The pH range for growth is between 5.0 and 10.0, with an optimum of pH 7.0–8.0. Growth occurs in the absence of NaCl and no growth occurs in 1.0 % (w/v) NaCl. Sensitive to 10 µg ml⁻¹ each of rifampicin and tetracycline and resistant to 10 µg ml⁻¹ each of kanamycin and streptomycin and 100 µg ampicillin ml⁻¹. Catalase- and oxidase-positive. Can hydrolyse casein, gelatin, elastin and cellulose but not guanine, hippurate, adenine, aesculin (in API 20 NE), starch or Tween 80. Can use L-aspartic acid 4-nitroanilide, Tween 40 and capric acid (in API 20 NE) as a single carbon source. In the Biolog GN2 kit, cannot use the following as single carbon sources: α-cyclodextrin, dextrin, glycogen, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, myo-inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, pyruvic acid, methyl ester, 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, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine,

L-asparagine, L-aspartic acid, glycyl L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyrogutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, potassium 5-ketogluconate, sodium malonate, potassium gluconate, capric acid, adipic acid, malic acid, citrate, palatinose, and L-tryptophan, while L-glutamic acid, glycyl L-glutamic acid and γ-aminobutyric acid are weakly positive. In ID 32 E tests, acid is not produced from glucose, inositol, trehalose, D-arabitol, mannitol, maltose, adonitol, sorbitol, rhamnose, palatinose, malonate, galacturonate, 5-ketogluconate, phenol red, L-arabitol, cellobiose, sucrose or L-arabinose, and no enzymic activity against 5-bromo-4-chloro-3-indolyl N-acetyl-β-D-glucosaminide, 5-bromo-3-indoxyl-nonanoate, 4-nitrophenyl α-D-glucopyranoside, 4-nitrophenyl α-D-maltopyranoside, 4-nitrophenyl β-D-glucopyranoside, 4-nitrophenyl β-D-glucuronide and 4-nitrophenyl α-D-galactopyranoside. Also, urea is not hydrolysed, indole is not produced and nitrate is not reduced to nitrite or nitrogen gas. In the API 20E kit, no acid production from any substrate or enzymic activity except gelatinase. Acetoin and H₂S are not produced from sodium pyruvate and sodium thiosulfate, respectively. In the API ZYM kit, enzymic activities for alkaline phosphatase, esterase (C-4), esterase lipase (C-8), lipase (C-14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsinase, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but not for α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Q-8 is the major quinone. Phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol are the major polar lipids. The DNA G + C content of the type strain is 65.8 mol%.

The type strain, YC6267^T (=KCTC 22247^T =DSM 21050^T), was isolated from a field of rice (*Oryza sativa* L.) managed under a no-tillage regime in Jinju, Korea.

Description of *Arenimonas composti* comb. nov.

Basonym: *Aspromonas composti* Jin *et al.* 2007.

The description is the same as that provided by Jin *et al.* (2007) for *Aspromonas composti*. In addition, it can hydrolyse hippurate but not elastin. The major fatty acid profile is similar to the previously published data except that small amounts of the cellular fatty acids iso-C_{15:0} 2-OH, C_{12:1} AT 11-12 and C_{10:0} are detected on R2A agar, but not on TSA. The type strain is TR7-09^T (=KCTC 12666^T =DSM 18010^T).

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