Lysobacter oryzae sp. nov., isolated from the rhizosphere of rice (*Oryza sativa* L.)

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The taxonomic position of a novel bacterial strain, YC6269^T, isolated from the rhizosphere of rice (*Oryza sativa* L.) managed under no-tillage practice in Jinju, South Korea, was studied using polyphasic approach. Cells of the strain were Gram-negative, rod-shaped and facultatively anaerobic. The novel strain grew at a temperature of 15–42 °C (optimum at 28 °C). Growth of the strain occurred between pH 5.5 and 11.0, with an optimum at pH 7.0–8.0. The G + C content of the total DNA was 67.4 mol%. The 16S rRNA gene sequence of the strain was most closely related to species of the genus *Lysobacter*, *Lysobacter yangpyeongensis* DSM 17635^T (98.6%), *Lysobacter niabensis* GH34-4^T (97.2%), *Lysobacter enzymogenes* DSM 2043^T (96.9%), *Lysobacter daejeonensis* DSM 17634^T (96.3%) and *Lysobacter niastensis* GH41-7^T (96.2%). The novel strain showed <96.0% similarity with other species of the genus *Lysobacter*. Chemotaxonomic data (major quinone, Q-8; major polar lipids, phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-*N*-methylethanolamine, and major fatty acids, C_{15:0} iso, C_{16:0} iso, C_{17:0} iso and C_{17:1} iso ω9c) supported the affiliation of strain YC6269^T to the genus *Lysobacter*. Phylogenetic analysis based on the 16S rRNA gene sequences, DNA–DNA hybridization data and biochemical and physiological characteristics strongly supported the genotypic and phenotypic differentiation of strain YC6269^T from recognized species of the genus *Lysobacter*. Strain YC6269^T, therefore, represents a novel member of the genus *Lysobacter*, for which the name *Lysobacter oryzae* sp. nov. is proposed. The type strain is YC6269^T (=KCTC 22249^T=DSM 21044^T).

The genus *Lysobacter*, grouped in the family *Xanthomonadaceae* belongs to the class *Gammaproteobacteria*. Species of the genus *Lysobacter* were originally grouped with myxobacteria because they shared the distinctive trait of gliding motility, but they display a number of unique traits that distinguish them from other taxonomically and ecologically related microbes, including high genomic DNA G + C content (typically ranging between 65.4 and 70.1 mol%) and a lack of flagella (Christensen & Cook, 1978). Interest in members of the *Lysobacter* group has increased recently as they have potential for the development of biocontrol agents against plant fungal pathogens (Folman *et al.*, 2004; Islam *et al.*, 2005; Kilic-Ekici & Yuen, 2003; Nakayama *et al.*, 1999; Park *et al.*, 2008) and antibiotic compounds

against human pathogens (Ahmed *et al.*, 2003; Chohnan *et al.*, 2002; Hashizume *et al.*, 2004; Kato *et al.*, 1998).

Species of the genus *Lysobacter* have been described as ubiquitous inhabitants of soil and water and are commonly found in diverse geographical and environmental habitats (Christensen & Cook, 1978; J. W. Lee *et al.*, 2006; Lueders *et al.*, 2006; Romanenko *et al.*, 2008; Weon *et al.*, 2006, 2007; Yassin *et al.*, 2007). Recently, their relevance to agriculture has been recognized and their roles as members of significant microbial communities associated with soil and plants have been explored (Folman *et al.*, 2001; Islam *et al.*, 2005; J. W. Lee *et al.*, 2006; M. S. Lee *et al.*, 2006; Park *et al.*, 2008).

During the analysis of the bacterial communities of two rice fields managed under conventional and no-tillage practices, many unknown strains were isolated by using a modified culture method and an improved isolation methodology. Some novel strains which belong to the genus *Lysobacter* were isolated by the improved culture method from the rhizosphere of rice at different growth

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YC6269^T is EU376963.

A transmission electron micrograph of cells of strain YC6269^T and the TLC polar lipid analysis are available as supplementary figures with the online version of this paper. Additional tables detailing the enzymic activities of the novel strain and the fatty acid profile are also available.

phases from the no-tillage field, but not from the conventional tillage field.

The improved culture method was based on a combination of factors such as longer incubation, sonication, selection of microcolonies with the help of magnifying lens, increasing the number of inoculation plates of each dilution, using modified half-strength R2A agar and some additional modifications (Hamaki *et al.*, 2005; Janssen *et al.*, 2002; Joseph *et al.*, 2003). The modified half-strength R2A agar consisted of 0.25 g yeast extract, 0.25 g proteose peptone No. 3 (Difco), 0.25 g Casamino acid, 0.25 g glucose, 0.25 g soluble starch, 0.15 g sodium pyruvate, 0.15 g K₂HPO₄, 0.03 g MgSO₄, 25 % soil extract (v/v) and 15 g agar in 1 l of distilled water. Three soil samples were collected from the rhizosphere of the rice from each field at three growth phases, vegetative, reproductive and ripening, from Gyeongsang National University farm (Daegok valley) located in the north-east of Jinju, Korea, during 2007. One sample was collected at the zero-phase (pre-sowing) from both fields. The rhizosphere samples were collected at a depth of 15–20 cm and from an area 6–10 cm wide from five randomly selected points in each field after the removal of the upper 3–5 cm soil, some 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 longer than one month. Single colonies on the plates were purified by transferring them onto new plates and these were incubated again on half-strength R2A (without soil extract) agar. One novel bacterial strain, YC6269^T, was isolated from the rhizosphere sample collected at the ripening phase from the no-tillage field. The novel strain was identified on the basis of 16S rRNA gene sequences, DNA–DNA hybridization data and other chemotaxonomic and genotypic characteristics (Park *et al.*, 2008). It was confirmed that this new strain represents a novel species in the genus *Lysobacter*. The novel strain was routinely cultured on half-strength R2A agar at 28 °C and maintained as a glycerol suspension (15 %, w/v) in half-strength R2A broth at –70 °C.

Cell morphology was observed using light (Nikon; × 1000) and transmission electron microscopy (model H-600; Hitachi) with cells grown for 1 day at 28 °C in half-strength R2A broth. Catalase and oxidase tests were performed by the procedures as outlined by Cappuccino & Sherman (2002). The physiological properties of strain YC6269^T were determined using previously described tests for 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 (Ten *et al.*, 2004) and Tween 20 (Atlas, 1993). Enzyme activities and acid production from different carbohydrates were determined by using API ZYM and API 20E kits, respectively, at 37 °C according to the manufacturer's instructions (bioMérieux). API ZYM strips were read after 5 h incubation. The assimilation of single carbon substrates was determined by using ID 32E

and API 20NE kits at 30 °C after 24 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) on 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 incubation. Salt tolerance was tested in half-strength R2A broth medium supplemented with 1–10 % (w/v) NaCl after 7 days incubation. Duplicate antibiotic sensitivity tests were performed using filter-paper discs containing the following: tetracycline, kanamycin, ampicillin, streptomycin and rifampicin, each with 10, 50 and 100 µg ml⁻¹ concentrations. Discs were placed on half-strength R2A agar plates spread with strain YC6269^T and reference strains and the plates were then incubated at 28 °C for 3 days. Almost all tests were performed with closely related reference strains including *Lysobacter antibioticus* KCTC 12129^T, *Lysobacter daejeonensis* DSM 17634^T, *Lysobacter enzymogenes* KCTC 12131^T, *Lysobacter gummosus* KCTC 12132^T, *Lysobacter koreensis* KCTC 12204^T, *Lysobacter niabensis* GH34-4^T and *Lysobacter yangpyeongensis* DSM 17635^T.

Extraction of genomic DNA was performed using a commercial genomic DNA extraction kit (Core Biosystem). The 16S rRNA gene of strain YC6269^T was PCR amplified from a small amount (0.5 µl) of purified genomic DNA by using a set of primers 27F and 1492R (Lane, 1991). The PCR product obtained was purified and sequenced according to Chung *et al.* (1999). The 16S rRNA gene sequences were compiled using SeqMan software (DNASTAR) and the sequences of related taxa were obtained from the GenBank database. The multiple alignments were performed by CLUSTAL_X program (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic trees were constructed by using a neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) in the MEGA4 program (Tamura *et al.*, 2007) with bootstrap values based on 1000 replications (Felsenstein, 1985).

For the measurement of the G + C content of chromosomal DNA, the genomic DNA of the novel strain 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 a reverse-phase HPLC. Cellular fatty acids were analysed using samples of *Lysobacter* species 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). The fatty acids analysed by GC (Hewlett Packard 6890) were identified using the Microbial Identification software package. The quinone system and the polar lipids were determined by TLC, as described previously (Lechevalier *et al.*, 1977; Tindall, 1990).

DNA–DNA hybridization was carried out to evaluate the genomic DNA relatedness between strain YC6269^T and the most closely related species of the genus *Lysobacter* (as determined by 16S rRNA gene sequence similarity of >97.0 %) to strain YC6269^T (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). It was performed with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using a FLX 800 microplate fluorescence reader (Bio-Tek) for fluorescence measurements. The hybridization temperature was 65.3 °C and reciprocal experiments were performed with strain YC6269^T, *L. yangpyeongensis* DSM 17635^T and *L. niabensis* GH34-4^T. Hybridization was conducted with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the remaining three values were utilized in the calculation of similarity values. The DNA relatedness values quoted are expressed as the means of these three values.

The novel strain was Gram-negative, non-motile and had gliding activity. Cells were short, rod-shaped (0.3–0.5 × 1.8–2.0 µm; see Supplementary Fig. S1, available in IJSEM Online) and occurred singly and in pairs. Colonies grown on half-strength R2A agar plates for 3 days were smooth, circular, pale yellow, 1–2 mm in diameter and changed form as they grew older due to the gliding motility. Fruiting bodies were not observed. Growth was facultatively anaerobic. The novel strain grew well on R2A agar, nutrient agar, 1.0 % trypticase soy broth (w/v) (plus 1.5 % agar) and eosin methylene blue (EMB) agar, but did not grow on MacConkey agar, full-strength trypticase soy agar or LB agar. All culture media were from Difco. The physiological characteristics of strain YC6269^T are summarized in the species description and a comparison of selective characteristics with related type strains is presented in Table 1. Enzymic activities are given in the species description and a comparison of these with closely related type strains of the genus *Lysobacter* is given in Supplementary Table S1 (available in IJSEM Online).

The cellular fatty acid profiles of strain YC6269^T and related type strains of the genus *Lysobacter* are shown in Supplementary Table S2 (see IJSEM Online). The major cellular fatty acids for strain YC6269^T included C_{17:1} iso ω9c (21.5 %), C_{16:0} N alcohol (16.3 %), C_{15:0} iso (12.5 %), C_{17:0} iso (12.3 %), C_{16:0} iso (8.5 %), C_{15:1} iso AT 5 (3.9 %), C_{11:0} iso (3.9 %), C_{16:1}ω7c alcohol (3.5 %), C_{11:0} iso 3-OH (3.2 %), C_{14:0} (2.8 %), C_{16:0} (2.7 %), C_{15:0} anteiso (2.2 %), C_{16:1}ω11c (2.1 %), C_{15:0} iso 3-OH (1.9 %), summed feature 3 (1.1 %) and trace amounts (<1.0 %) of some other fatty acids were detected. Although the major fatty acids were similar to those found for other members of the genus *Lysobacter*, differences were found between some species in the genus *Lysobacter* and the novel strain. Fatty acids C_{16:0} N alcohol and C_{15:0} iso 3-OH were detected only in strain YC6269^T (Supplementary Table S1). The following polar lipids were present: phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl-N-methylethanolamine and some unknown amino group-containing

lipids (see Supplementary Fig. S2 in IJSEM Online), demonstrating the relatedness of the novel strain to the genus *Lysobacter* (Park *et al.*, 2008).

The DNA G + C content of strain YC6269^T was 67.4 mol%. This value is within the range for the genus *Lysobacter* (Christensen & Cook, 1978). The major quinone for the novel strain was Q-8 and this quinone has also been reported as the major quinone for all of the type strains of recognized members of the genus *Lysobacter*.

The 16S rRNA gene sequence of strain YC6269^T was a continuous stretch of 1415 bp. Sequence similarity calculations after a neighbour-joining analysis indicated that the closest relatives of the novel strain were *L. yangpyeongensis* DSM 17635^T (98.6 %), *L. niabensis* GH34-4^T (97.2 %), *L. enzymogenes* DSM 2043^T (96.9 %), *L. daejeonensis* DSM 17634^T (96.3 %) and *L. niastensis* GH41-7^T (96.2 %). Other species of the genus had low similarity values of <96.0 %. The phylogenetic trees based on the neighbour-joining and maximum-parsimony methods (Fig. 1) showed that strain YC6269^T formed a cluster with the closely related species of the genus *Lysobacter*, supported by a high bootstrap value (89 %). DNA–DNA hybridization levels between strain YC6269^T and *L. yangpyeongensis* DSM 17635^T and *L. niabensis* GH34-4^T were 45.0 and 18.0 %, respectively, which revealed that strain YC6269^T belongs to a distinct genomic species (Wayne *et al.*, 1987; Stackebrandt and Goebel, 1994).

Low levels of DNA–DNA hybridization, phylogenetic analysis (Fig. 1), enzymic activities, differences in other physiological and biochemical characteristics (Table 1 and Supplementary Table S1), antibiotic-sensitivity and the fatty acid profile (Supplementary Table S2) clearly distinguish strain YC6269^T from all the recognized species of the genus *Lysobacter*. Thus, on the basis of the evidence given above, we propose that strain YC6269^T represents the type strain of a novel species of the genus *Lysobacter*, *Lysobacter oryzae* sp. nov.

Description of *Lysobacter oryzae* sp. nov.

Lysobacter oryzae (o.ry'zae. L. gen. n. *oryzae* of rice, pertaining to the isolation of the type strain from the rhizosphere of rice).

Cells are Gram-negative, facultatively anaerobic, short rod-shaped, non-spore-forming, non-motile (but have gliding activity) and are 0.3–0.5 µm wide × 1.8–2.0 µm long. Cells occur singly and in pairs. Colonies grown on half-strength R2A agar at 28 °C for 3 days are 1–2 mm in diameter, pale yellow, smooth and circular. The temperature range for growth is 15–42 °C, optimum temperature for growth is 28 °C. The pH growth range is between 5.5 and 11.0, with an optimum of pH 7.0–8.0. Growth occurs in the absence of NaCl in half-strength R2A medium and no growth occurs in 1.0 % (w/v) NaCl. Resistant to 10 µg ml⁻¹ of tetracycline, kanamycin, streptomycin and rifampicin, but sensitive to 10 µg ml⁻¹ ampicillin. Catalase- and oxidase-

Table 1. Differential phenotypic characteristics between strain YC6269^T and related species of the genus *Lysobacter*

Taxa: 1, strain YC6269^T; 2, *L. yangpyeongensis* DSM 17635^T; 3, *L. niabensis* GH34-4^T (data from Weon *et al.*, 2007); 4, *L. koreensis* Dae16^T; 5, *L. daejeonensis* DSM 17634^T; 6, *L. gummosus* KCTC 12132^T; 7, *L. antibioticus* KCTC 12129^T; 8, *L. enzymogenes* KCTC 12131^T. All data are from this study unless otherwise indicated. ND, Not determined; +, positive; ±, weak; –, negative. All of the strains tested are able to hydrolyse casein and gelatin, but not agar or urea.

Characteristic	1	2	3	4	5	6	7	8
Cell size (µm)	0.3–0.5 × 1.8–2.0	0.4–0.6 × 3.0–4.0 ^a	0.5 × 2.0–5.0	0.5–0.8 × 1.5–2.0 ^b	0.4–0.6 × 3.0–4.0 ^a	0.4 × 2.0 ^a	0.4 × 6.5 ^a	0.5 × 38.0 ^a
Colony type	Smooth	Smooth	Irregular	Smooth	Smooth	Smooth	Mucoid	Smooth
Colony colour†	PY	Y	Y	Y	Y	PYG	C	DY–C
Aerobic/facultatively anaerobic‡	F	A	A	A	A	F	F	F
NaCl concentration for growth (% w/v):								
Optimum	0	0 ^a	0	0 ^b	0 ^a	0 ^c	0 ^c	0 ^c
Range	0	0–1.0 ^a	0–1.0	0–2.0 ^b	0–3.0 ^a	0–2.0 ^c	0–1.0 ^c	0–1.0 ^c
No growth	1.0	2.0 ^a	2.0	3.0 ^b	ND ^a	3.0 ^c	3.0 ^c	3.0 ^c
Catalase	+	–	+	+	–	+	+	+
Oxidase	+	+	+	–	+	+	+	+
API ZYM kit:								
α-Chymotrypsin	+	–	±	–	–	–	–	+
Trypsin	+	+	–	–	+	–	–	+
α-Glucosidase	+	+	–	–	+	–	–	+
N-Acetyl-β-glucosaminidase	–	+	+	–	–	+	+	–
Hydrolysis of:								
Chitin	–	–	–	–	–	+	+	+
Starch	–	+	+	–	–	–	–	–
Tween 20	–	+	ND	+	+	+	+	+
Growth on/at:								
Citrate	–	–	–	+	–	+	+	+
D-Glucose	–	–	–	–	+	+	–	+
Arabinose	–	–	–	+	–	+	–	–
Maltose	–	–	–	–	+	+	+	+
pH 4	–	–	–	ND	–	+	–	±
pH 10	+	–	–	ND	–	–	–	+
MacConkey agar	–	–	–	–	–	–	–	+
EMB agar§	+, PI	–	–	+, PI	–	+, PI	+, PI	+, PI
DNA G + C content (mol%)	67.4	67.3 ^a	62.5	68.9 ^b	61.7 ^a	65.7 ^c	69.2 ^c	69.0 ^c

*Data taken from other studies as indicated: ^aWeon *et al.* (2006); ^bJ. W. Lee *et al.*, 2006; ^cChristensen & Cook (1978).

†DY, deep yellow; PYG, pale yellowish-grey; PY, pale yellow; Y, yellow; C, cream.

‡A, Aerobic; F, facultative anaerobic.

§Colour of growth indicated as: PI, pink. EMB, Eosin methylene blue.

positive. Hydrolyses casein, hippurate and gelatin but does not hydrolyse elastin, guanine, adenine, aesculin, starch or Tween 20. Utilizes 5-bromo-3-indoxyl-nonanoate, 4-nitrophenyl α-D-glucopyranoside and 4-nitrophenyl-α-D-maltopyranoside as a single carbon source but not the following: L-arabitol, galacturonic acid, potassium 5-ketogluconate, sodium pyruvate, D-mannitol, maltose, potassium gluconate, capric acid, adipic acid, malic acid, citrate, phenylacetic acid, adonitol, palatinose, L-tryptophan, 5-bromo-4-chloro-3-indolyl-N-acetyl-β-D-glucosaminide, 4-nitrophenyl-β-D-glucopyranoside, 4-nitrophenyl-β-D-glucuronide, 4-nitrophenyl-α-D-galactopyranoside, sodium malonate, D-glucose, D-mannose, sucrose,

L-arabinose, D-arabitol, trehalose, D-rhamnose, inositol, cellobiose or D-sorbitol. Acid is not produced from glucose, inositol, D-mannitol, sorbitol, rhamnose, melibiose, amygdalin, sucrose or L-arabinose. Urea is not hydrolysed. Indole and H₂S are not produced. Acetoin is produced. Nitrate is not reduced to nitrite or nitrogen gas. Positive in tests for activities of alkaline phosphatase, esterase (C-4), esterase lipase (C-8), leucine arylamidase, valine arylamidase, trypsinase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, but not for lipase (C-14), cystine arylamidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. Q-8 is

Fig. 1. Phylogenetic tree constructed from comparative analysis of 16S rRNA gene sequences showing the relationships between strain YC6269^T and related species of the genus *Lysobacter*. The tree was constructed by using the neighbour-joining method and Jukes & Cantor evolutionary distance matrix data obtained from aligned nucleotides. Solid circles indicate that the corresponding clades were also recovered in maximum-parsimony trees. Bootstrap values (expressed as percentage of 1000 replications) greater than 50% are shown at the branch points. Bar, 1 substitution per 100 nucleotide positions.

the major quinone. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-N-methylethanolamine.

The type strain, YC6269^T (=KCTC 22249^T=DSM 21044^T), was isolated from the rhizosphere of rice managed under no-tillage practice in Jinju, Korea. The DNA G+C content of the type strain is 67.4 mol%.

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