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Lysobacter niabensis sp. nov. and *Lysobacter niastensis* sp. nov., isolated from greenhouse soils in Korea

Hang-Yeon Weon,[1] Byung-Yong Kim,[2] Min-Kyeong Kim,[3] Seung-Hee Yoo,[2] Soon-Wo Kwon,[2] Seung-Joo Go[2] and Erko Stackebrandt[4]

Correspondence
Soon-Wo Kwon
swkwon@rda.go.kr

[1]Applied Microbiology Division, National Institute of Agricultural Science and Technology, Rural Development Administration (RDA), Suwon 441-707, Republic of Korea

[2]Korean Agricultural Culture Collection (KACC), Microbial Genetics Division, National Institute of Agricultural Biotechnology, RDA, Suwon 441-707, Republic of Korea

[3]Environment and Ecology Division, National Institute of Agricultural Science and Technology, RDA, Suwon 441-707, Republic of Korea

[4]Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

Two bacterial strains, designated GH34-4^T and GH41-7^T, were isolated from greenhouse soil cultivated with cucumber. The bacteria were strictly aerobic, Gram-negative, rod-shaped and oxidase- and catalase-positive. 16S rRNA gene sequence analysis indicated that these strains belong to the genus *Lysobacter* within the *Gammaproteobacteria*. Strain GH34-4^T showed highest sequence similarity to *Lysobacter yangpyeongensis* GH19-3^T (97.5 %) and *Lysobacter koreensis* Dae16^T (96.4 %), and strain GH41-7^T showed highest sequence similarity to *Lysobacter antibioticus* DSM 2044^T (97.5 %), *Lysobacter enzymogenes* DSM 2043^T (97.5 %) and *Lysobacter gummosus* ATCC 29489^T (97.4 %). Levels of DNA–DNA relatedness indicated that strains GH34-4^T and GH41-7^T represented species clearly different from *L. yangpyeongensis*, *L. antibioticus*, *L. enzymogenes* and *L. gummosus*. The major cellular fatty acids of strains GH34-4^T and GH41-7^T were iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega9c$, and the major isoprenoid quinone was Q-8. The DNA G+C contents of GH34-4^T and GH41-7^T were 62.5 and 66.6 mol%, respectively. On the basis of the polyphasic taxonomic data presented, it is evident that each of these strains represents a novel species of the genus *Lysobacter*, for which the names *Lysobacter niabensis* sp. nov. (type strain GH34-4^T=KACC 11587^T=DSM 18244^T) and *Lysobacter niastensis* sp. nov. (type strain GH41-7^T=KACC 11588^T=DSM 18481^T) are proposed.

The genus *Lysobacter* was first proposed by Christensen & Cook (1978) to accommodate non-fruiting, gliding bacteria with a high G+C content. Three novel species of the genus, *Lysobacter koreensis* (Lee *et al.*, 2006), *Lysobacter daejeonensis* and *Lysobacter yangpyeongensis* (Weon *et al.*, 2006), isolated from soils, have recently been described. At the time of writing, the genus *Lysobacter* comprises eight species with validly published names, *Lysobacter enzymogenes*, *L. antibioticus*, *L. brunescens*, *L. concretionis*, *L. daejeonensis*, *L. gummosus*, *L. koreensis* and *L. yangpyeongensis*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains GH34-4^T and GH41-7^T are respectively DQ462461 and DQ462462.

A table giving the fatty acid compositions of strains GH34-4^T and GH41-7^T is available as supplementary material in IJSEM Online.

In 2005, soil samples were collected from greenhouse soil cultivated with cucumber (*Cucumis sativus* L.) from the Yongin and Sanju regions, Korea. The samples were serially diluted with 0.85 % NaCl (w/v) and suitable 10-fold dilutions were plated onto R2A agar (Difco). The plates were incubated at 28 °C for 4 days and strains GH34-4^T and GH41-7^T were subsequently isolated.

For strains GH34-4^T and GH41-7^T, cell morphology was determined by using phase-contrast microscopy of 2-day-old cultures. Gliding motility was observed via oil-immersion phase-contrast microscopy of the edge of colonies in exponential growth phase. The temperature range (5–50 °C), pH range (pH 4–10 at intervals of 1 pH unit) and requirement for 0, 1, 2, 3, 5 or 7 % NaCl (w/v) were determined by using R2A medium. Tests for

Gram staining, catalase, oxidase and hydrolysis of casein, DNA and starch were conducted according to the methods of Smibert & Krieg (1994). Tests were also made for hydrolysis of CM-cellulose (0.1 %, w/v), chitin from crab shells (1 %, w/v) and tyrosine (0.5 %, w/v). The commercially available API 20NE and API ID 32 GN (bioMérieux) systems were used to determine biochemical properties, utilization of carbohydrates and enzyme activities according to the manufacturer's instructions. The API ZYM tests were read after 4 h incubation at 37 °C, and other API tests after 72 h at 28 °C.

Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High prime system (Roche). Reassociation was conducted at 60 °C. Hybridized DNAs were visualized using the DIG luminescent detection kit (Roche). Levels of DNA–DNA relatedness were quantified by using a densitometer (Bio-Rad). For fatty acid methyl ester analysis, cell mass was harvested from R2A agar after cultivation for 48 h at 28 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Determination of DNA G+C contents was performed according to Mesbah *et al.* (1989) by using a reversed-phase column (Supelcosil LC-18-S; Supelco).

The 16S rRNA gene was amplified from colonies by PCR using primers fD1 and rP2 (Weisburg *et al.*, 1991) and the entire PCR fragment was directly sequenced (Hiraishi, 1992). The 16S rRNA gene sequences were aligned by using the MEGALIGN program of DNASTAR. A phylogenetic tree was reconstructed with the neighbour-joining method of Saitou & Nei (1987) on MEGA version 2.1 (Kumar *et al.*, 2001). The stability of relationships was assessed by performing bootstrap analyses of the neighbour-joining data based on 1000 resamplings.

Strains GH34-4^T and GH41-7^T were aerobic, Gram-negative, rod-shaped and catalase- and oxidase-positive. They grew well on R2A, trypticase soy agar (Difco) and nutrient agar (Difco), but did not grow on MacConkey agar (Difco). The phenotypic characteristics of strains GH34-4^T and GH41-7^T are given in Table 1 and in the species descriptions below. Differential properties among GH34-4^T, GH41-7^T and recognized species of the genus *Lysobacter* are given in Table 1.

Strains GH34-4^T and GH41-7^T had ubiquinone-8 (Q-8) as the major isoprenoid quinone, which is a characteristic feature of the genus *Lysobacter* (Bae *et al.*, 2005). The fatty acid compositions of the novel strains and closely related *Lysobacter* species are given in Supplementary Table S1 available in IJSEM Online. The major fatty acids detected (percentages of the total cellular fatty acids) from strains GH34-4^T and GH41-7^T were iso-$C_{16:0}$ (23.7 and 23.3 %, respectively), iso-$C_{15:0}$ (12.7 and 21.9 %) and iso-$C_{17:1}\omega 9c$ (10.0 and 10.9 %). The DNA G+C contents of strains GH34-4^T and GH41-7^T were 62.5 and 66.6 mol%, respectively.

The phylogenetic tree using the almost-complete 16S rRNA gene sequence (approximately 1450 bp) of strains GH34-4^T and GH41-7^T showed clearly that the two strains were located within the genus *Lysobacter* (Fig. 1). Strain GH34-4^T was most closely related to *L. yangpyeongensis* GH19-3^T (97.5 % 16S rRNA gene sequence similarity). Strain GH41-7^T was grouped with several *Lysobacter* species, showing highest sequence similarity to *L. antibioticus* DSM 2044^T (97.5 %), *L. enzymogenes* DSM 2043^T (97.5 %) and *L. gummosus* ATCC 29489^T (97.4 %). The level of DNA–DNA relatedness between strain GH34-4^T and *L. yangpyeongensis* GH19-3^T was 25 %, and levels between strain GH41-7^T and the type strains of *L. enzymogenes*, *L. antibioticus* and *L. gummosus* were 42, 39 and 32 %, respectively.

On the basis of the phenotypic, chemotaxonomic and genetic data presented, it is proposed that strains GH34-4^T and GH41-7^T should be placed in the genus *Lysobacter* as the type strains of novel species, with the names *Lysobacter niabensis* sp. nov. and *Lysobacter niastensis* sp. nov., respectively.

Description of *Lysobacter niabensis* sp. nov.

Lysobacter niabensis (ni.ab.en′sis. N.L. masc. adj. *niabensis* pertaining to NIAB, National Institute of Agricultural Biotechnology, where taxonomic studies of this taxon were conducted).

Cells are aerobic, Gram-negative rods (0.5 × 2.0–5.0 μm). Growth occurs at 5–37 °C (optimum 28 °C), pH 5–8 (optimum pH 6–7) and 0–1 % NaCl. Colonies are yellow and irregular after 48 h of cultivation at 28 °C on R2A medium. Casein, starch and tyrosine are hydrolysed, but chitin, CM-cellulose, DNA and urea are not. Major cellular fatty acids are iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega 9c$. The detailed cellular fatty acid composition is given in Supplementary Table S1 available in IJSEM Online. The major isoprenoid quinone is Q-8. The DNA G+C content of the type strain is 62.5 mol%.

The type strain, GH34-4^T (=KACC 11587^T=DSM 18244^T), was isolated from greenhouse soil in the Republic of Korea.

Description of *Lysobacter niastensis* sp. nov.

Lysobacter niastensis (ni.as.ten′sis. N.L. masc. adj. *niastensis* pertaining to NIAST, National Institute of Agricultural Science and Technology, where taxonomic studies of this taxon were conducted).

Cells are aerobic, Gram-negative rods (0.5–0.6 × 2.0–4.0 μm). Growth occurs at 10–40 °C (optimum 28 °C), pH 4–9 (optimum pH 6–8) and 0–1 % NaCl. Motile by gliding. Colonies are light beige, convex, round with clear margins after 48 h of cultivation at 28 °C on R2A medium.

Table 1. Differential phenotypic characteristics of strains GH34-4^T and GH41-7^T and recognized *Lysobacter* species

Strains: 1, strain GH34-4^T; 2, strain GH41-7^T; 3, *L. antibioticus* DSM 2044^T; 4, *L. brunescens* DSM 6979^T; 5, *L. concretionis* DSM 16239^T; 6, *L. daejeonensis* KACC 11406^T; 7, *L. enzymogenes* DSM 2043^T; 8, *L. gummosus* DSM 6980^T; 9, *L. koreensis* KCTC 12204^T; 10, *L. yangpyeongensis* KACC 11407^T. Data are taken from Swings & Christensen (1989), Bae *et al.* (2005), Lee *et al.* (2006), Weon *et al.* (2006) and this study. According to the API 20NE test strips, all strains are positive for gelatin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. According to the API 20NE and API ID 32GN test strips, all strains are unable to assimilate L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, phenylacetic acid, L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-fucose, D-sorbitol, propionic acid, potassium 2-ketogluconate or 4-hydroxybenzoic acid. +, Positive; W, weakly positive; −, negative; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell size (μm)	0.5 × 2.0–5.0	0.5–0.6 × 2.0–4.0	0.4 × 6.5	0.3 × 11.0	0.7 × 1.0–13.5	0.4–0.6 × 3.0–4.0	0.5 × 38.0	0.4 × 2.0	0.5–0.8 × 1.5–2.0	0.4–0.6 × 3.0–4.0
Gliding motility	−	+	+	+	+	−	+	+	ND	−
Catalase/oxidase	+/+	+/+	+/+	+/+	+/+	W/+	+/+	+/ND	+/−	−/+
API 20NE:*										
Nitrate reduction	−	+	+	−	+	+	−	−	−	−
Aesculin hydrolysis	−	+	+	+	−	+	+	+	−	−
β-Galactosidase	−	+	+	−	−	−	+	+	−	−
Assimilation (API 20NE and API ID 32GN) of:*										
D-Glucose	−	W	+	−	−	+	+	+	−	−
D-Mannose	−	−	+	−	−	−	+	+	−	−
N-Acetylglucosamine	−	+	+	−	−	−	+	+	−	−
D-Maltose	−	+	+	−	−	+	+	+	−	−
Malic acid	−	−	+	−	−	−	+	+	−	−
Trisodium citrate	−	−	−	−	−	−	+	−	−	−
Sucrose	−	−	−	−	−	−	+	+	−	−
Sodium acetate	−	−	+	−	+	+	W	W	−	−
Glycogen	−	−	+	−	W	+	+	+	−	+
L-Serine	−	−	+	−	−	−	+	+	−	−
Salicin	−	−	−	−	−	−	+	+	−	−
D-Melibiose	−	−	−	−	−	−	+	+	−	−
Valeric acid	−	−	+	−	+	+	+	W	−	−
L-Histidine	−	+	+	−	−	−	W	−	−	−
3-Hydroxybutyric acid	−	−	+	−	+	+	+	+	−	+
L-Proline	−	−	+	−	+	−	+	+	−	−
DNA G+C content (mol%)	62.5	66.6	69.2	67.7	63.8	61.7	69.0	65.7	68.9	67.3

*Data from the present study except those for *L. daejeonensis* KACC 11406^T and *L. yangpyeongensis* KACC 11407^T.

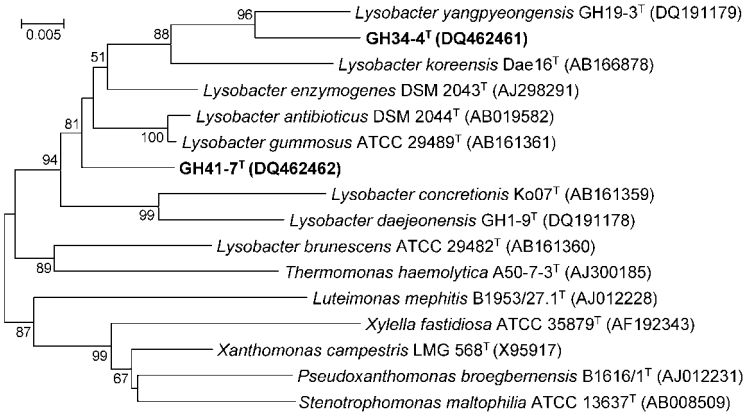

Fig. 1. Phylogenetic tree constructed on the basis of 16S rRNA gene sequence analysis of strains GH34-4^T and GH41-7^T and their relatives. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 re-sampled datasets. Only values >50 % are indicated. Bar, 0.5 substitutions per 100 nt.

Casein, starch and tyrosine are hydrolysed, but chitin, CM-cellulose, DNA and urea are not. Major cellular fatty acids are iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega 9c$. The detailed cellular fatty acid composition is given in Supplementary Table S1 available in IJSEM Online. The major isoprenoid quinone is Q-8. The DNA G+C content of the type strain is 66.6 mol%.

The type strain, GH41-7^T (=KACC 11588^T=DSM 18481^T), was isolated from greenhouse soil in the Republic of Korea.

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