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Niabella terrae sp. nov. Isolated from Greenhouse Soil[§]

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An orange-colored bacterial strain, ICM 1-15^T, was isolated from greenhouse soil. The 16S rRNA gene sequence of this strain showed the highest sequence similarity with *Niabella ginsengisoli* GR10-1^T (95.2%) and *Niabella yanshanensis* CCBAU 05354^T (95.0%) among the type strains. The strain ICM 1-15^T was a strictly aerobic, Gram-negative, non-spore-forming, non-motile, flexirubin pigment-producing, short rod-shaped bacterium. The strain grew at 15–35°C (optimum, 25°C), at a pH of 5.0–8.5 (optimum, pH 6.5), and in the presence of 0–3% NaCl (optimum, 1%). The DNA G+C content of strain ICM 1-15^T was 43.6 mol%. It contained MK-7 as the major isoprenoid quinone and iso-$C_{15:0}$ (38.9%), iso-$C_{15:1}$ G (20.3%), and iso-$C_{17:0}$ 3-OH (12.9%) as the major fatty acids. On the basis of evidence from our polyphasic taxonomic study, we concluded that strain ICM 1-15^T should be classified within a novel species of the genus *Niabella*, for which the name *Niabella terrae* sp. nov. is proposed. The type strain is ICM 1-15^T (=KACC 17443^T =JCM 19502^T).

Keywords: *Niabella terrae*, novel species, polyphasic taxonomy

Introduction

Bacteria of the genus *Niabella* are characterized as Gram-negative, strictly aerobic, flexirubin pigment-producing short rods. Phylogenetically, the genus belongs to the family *Chitinophagaceae* and the phylum *Bacteroidetes*. At the time of this writing, the genus comprised seven recognized species, *N. aurantiaca* (Kim *et al.*, 2007), *N. soli* (Weon *et al.*, 2008), *N. ginsengisoli* (Weon *et al.*, 2009), *N. yanshanensis* (Wang *et al.*, 2009), *N. tibetensis* (Dai *et al.*, 2011), *N. hirudinis*, and *N. drilacis* (Glaeser *et al.*, 2013). The first five species were isolated from soil while the last two were from bladders of the medical leech, *Hirudo verbena*. During the course of an investigation of a bacterial community in agricultural soils, one isolate was shown to represent a novel species of the genus *Niabella* on the basis of phenotypic data and phylogenetic inference.

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Materials and Methods

Bacterial strains

Strain ICM 1-15^T was isolated from a greenhouse soil cultivated with lettuce in Icheon, Korea (37°14.17′ N, 127°22.17′E). The soil sample had the following chemical properties: pH 6.6; total organic carbon, 23 g/kg; available P_2O_5, 1,184 mg/kg; exchangeable K, 0.45 cmol/kg; exchangeable Ca, 2.5 cmol/kg, and exchangeable Mg, 1.5 cmol/kg. The soil texture was sandy loam. The soil sample was diluted serially in a saline solution (0.85%, w/v), spread on R2A agar (Difco, USA), and incubated for 5 days at 28°C. Type strains of the genus *Niabella* were obtained from the Korean Agricultural Culture Collection (KACC, Korea) for comparative taxonomic analysis.

Phylogenetic analysis

The 16S rRNA gene was amplified by PCR using two universal primers, as described previously (Kwon *et al.*, 2003). The sequence of the amplified 16S rRNA gene was analyzed using an Applied Biosystems ABI 3100 DNA sequencer using the primers 800R (5′-TACCAGGGTATCTAATCC-3′), 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 984F (5′-AACGCGAAGAACCTTAC-3′). The nearly complete 16S rRNA gene sequence of strain ICM 1-15^T (1,450 nt) was obtained from the sequence fragments using the SeqMan software (DNASTAR) and imported into the ARB software package (version 5.5) (Ludwig *et al.*, 2004) along with the 16S rRNA gene sequences of the selected type strains belonging to the family *Chitinophagaceae*. These sequences were aligned using the SINA aligner (version 1.1) (Pruesse *et al.*, 2012) and the SILVA 16S rRNA database (SSURef-111) (Pruesse *et al.*, 2007) and exported to the MEGA program (version 5.1) (Tamura *et al.*, 2011). The maximum-likelihood and neighbor-joining trees were constructed using the positional variability filter for bacteria provided with the ARB package. Nucleotide similarity values were calculated using the EzTaxon-e server (Kim *et al.*, 2012).

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ICM 1-15^T is KF289904.

Determination of DNA G+C content

The DNA G+C content was determined as described by

Gonzalez and Saiz-Jimenez (2002) using the CFX96 system (Bio-Rad, USA).

Morphological, physiological, and biochemical characterization

The cell morphology and motility of strain ICM 1-15^T was examined by using oil-immersion phase-contrast microscopy (Axioplan 2; Zeiss, Germany) with cells grown for 3 days on R2A agar at 28°C. In addition to R2A agar, growth of strain ICM 1-15^T was tested on trypticase soy agar (TSA), nutrient agar (NA), Luria-Bertani agar (LB), and marine agar 2216 (MA). Growth in the presence of 0, 0.5, 1.0, 2.0, 3.0, and 4.0% NaCl (w/v), and at various temperatures (10–40°C at intervals of 5°C) was investigated after 7 days of incubation on R2A agar. The pH range for growth was determined after 7 days of incubation in R2A broth at a pH of 3.0–10.0 in increments of 0.5 units. The pH was adjusted by using the following buffer systems: citric acid/Na_2HPO_4 (pH 3.0–6.0), NaH_2PO_4/Na_2HPO_4 (pH 6.5–8.0), Tris/HCl (pH 8.5 and pH 9.0), and $Na_2CO_3/NaHCO_3$ (pH 9.5 and pH 10.0) (Gomori, 1955). Gram staining behavior was determined by the KOH test (Smibert and Krieg, 1994) and L-alanine aminopeptidase activity test (Bactident Aminopeptidase test kit; Merck, Germany). Activities of catalase and oxidase were measured and the ability to hydrolyse starch (1.0%, w/v), casein (10% skimmed milk, w/v), lipids (1.0% tributyrin), chitin (0.5%, w/v), and carboxy-methylcellulose (CM-cellulose, 0.1%, w/v) was examined as described by Smibert and Krieg (1994). Growth under anaerobic conditions was tested by incubating R2A agar plates in AnaeroGen sachet pouches (Oxoid, England) at 28°C for 2 weeks. Flexirubin-type pigments were revealed by the color shift that occurred after exposure of the colonies to a 20% (w/v) KOH solution (Reichenbach, 1992). Other biochemical characteristics were determined using the API 20NE, API ZYM, and API ID 32GN systems according to the instructions of the manufacturer (bioMérieux, France). The API ZYM test strip was read after a 4-h incubation at 37°C, while the other API strips were examined after 7 days at 28°C.

Chemotaxonomy

Cells were grown on R2A agar for 3 days at 28°C and fatty acid methyl esters were extracted and prepared according to the standard protocol of the Microbial Identification System (MIDI; Microbial ID, USA). Briefly, the method entailed (i) saponification of whole-cell preparations (40 mg of cells from plate culture) at 100°C with 1 ml of methanolic NaOH (15% [w/v] NaOH in 50% [v/v] methanol), (ii) esterification of the fatty acids at 80°C with 2 ml of 3.25 N HCl in 46% (v/v) methanol, (iii) extraction of the FAMEs into 1.25 ml of 1:1 (v/v) methyl-tert-butyl etherhexane, and (iv) aqueous washing of the organic extract with 3 ml of 1.2% (w/v) NaOH. The washed extracts were then analyzed on a gas chromatograph (Agilent Ultra 2 column; carrier gas, hy-

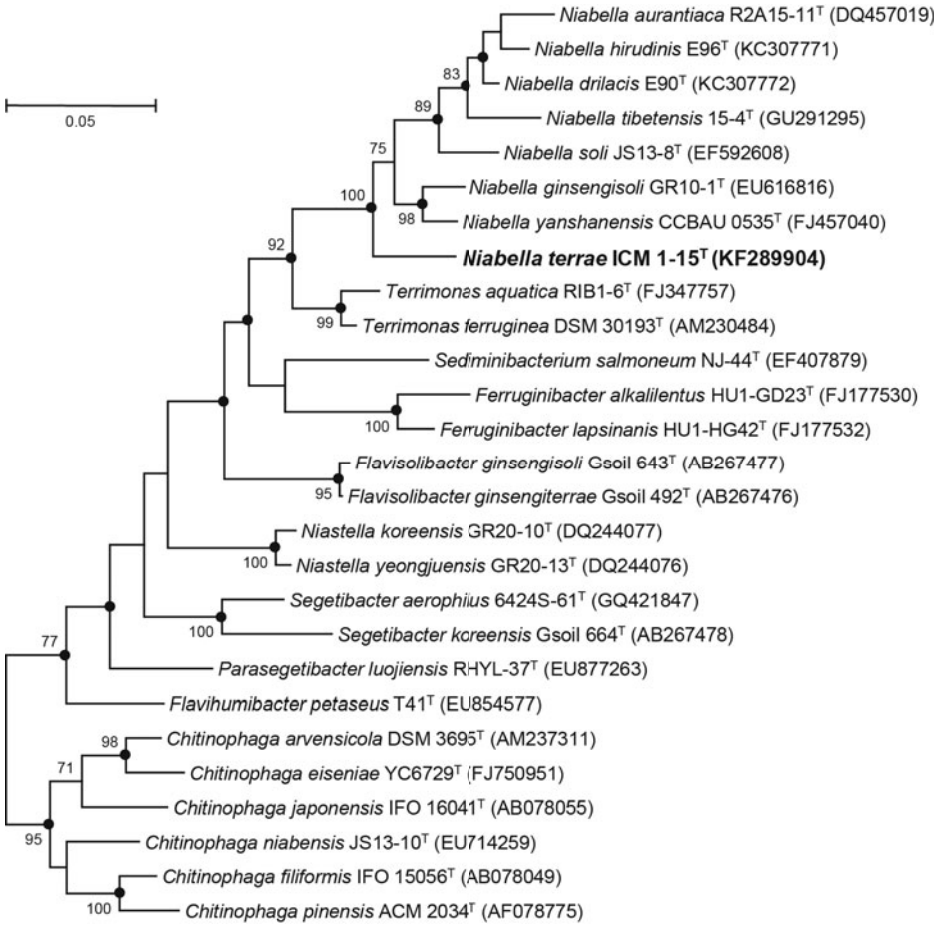

Fig. 1. Maximum-likelihood tree based on a comparative analysis of 16S rRNA gene sequences showing the relationship between strain ICM 1-15^T and the type strains belonging to the family *Chitinophagaceae*. Bootstrap values (>70%) based on 500 resamplings are shown at branching points. The dots indicate that the corresponding branches were also recovered in the neighbor-joining tree. The scale bar indicates 0.05 estimated change per nucleotide. *Lewinella nigricans* ATCC 2314^T was used as an outgroup (not shown in the tree).

drogen, temperature ramping from 120 to 260°C at a rate of 5°C/min) with a flame ionization detector. Individual fatty acids were identified using MIDI standards and TSBA6 database (version 6.10) (Microbial ID, USA).

The presence of isoprenoid quinones was investigated using high-performance liquid chromatography (HPLC), as described previously (Groth *et al.*, 1996). Isoprenoid quinone-containing extracts were dissolved in acetone and were applied as 4-cm bands to 20 × 20-cm TLC plates of aluminum-backed silica gel 60 F254 sheets. After development with hexane-ethyl ether (9:1, v/v), separated components were revealed using short wave (254 nm) ultraviolet light. Bands were marked with a pencil, cut from the plates, and extracted with 1-ml diethyl ether; the solvent was removed by evaporation under a stream of nitrogen. The extracts were resuspended in 200 µl isopropanol. The filtered samples were analyzed by reverse-phase HPLC.

Results and Discussion

Phylogenetic analysis

Analyses of the 16S rRNA gene sequences showed that strain ICM 1-15^T shared the highest sequence similarity with *Niabella ginsengisoli* GR10-1^T (95.2%) and *Niabella yanshanensis* CCBAU 05354^T (95.0%) among the type strains. The phylogenetic analysis based on the maximum-likelihood tree showed that the strain ICM 1-15^T formed a clade with the type strains of the genus *Niabella* with a 100% bootstrap value (Fig. 1), which was also supported by the neighbor-joining tree (Supplementary data Fig. S1).

Morphological, physiological, and biochemical characteristics

Following growth on R2A agar at 28°C for 5 days, strain ICM 1-15^T formed dark yellow-to-orange, circular (1.0–1.5 mm in diameter) convex colonies with entire margins. Cells of strain ICM 1-15^T were strictly aerobic, Gram-negative, non-spore-forming, non-motile short rods that were 0.4–0.6 µm wide and 0.6–1.1 µm long. In addition to R2A agar, strain ICM 1-15^T showed growth on TSA, NA, LB, and MA media. The temperature range for growth was 15–35°C with optimum growth at 25°C. The pH range for growth was 5.0–8.5 with optimum growth at pH 6.5. Cells were positive for catalase activity and produced flexirubin pigment. Other phenotypic characteristics of strain ICM 1-15^T and type strains of *Niabella* species are shown in Table 1. CM-cellulose was hydrolyzed only by strain ICM 1-15^T and *N. yanshanensis* KACC 14980^T. D-mannitol was assimilated only by strain ICM 1-15^T and *N. soli* KACC 12604^T, while

Table 1. Differentiation between strain strain ICM 1-15^T and the other type strains of *Niabella* species

Strains: 1, ICM 1-15^T; 2, *N. aurantiaca* KACC 11698^T; 3, *N. ginsengisoli* KACC 13021^T; 4, *N. soli* KACC 12604^T; 5, *N. tibetensis* KACC 15620^T; 6, *N. yanshanensis* KACC 14980^T. +, Positive; -, negative.

All strains are positive for hydrolysis of starch and esculin, β-galactosidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase but negative for nitrate reduction, arginine dihydrolase, urease, lipase (C14), crystine arylamidase, α-chymotrypsin, β-glucuronidase, and fermentation of D-glucose. All strains assimilate D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, L-rhamnose, D-saccharose, glycogen, salicin, D-melibiose, and L-arabinose but not assimilate gluconate, capric acid, adipic acid, malic acid, citrate, phenyl acetic acid, itaconic acid, suberic acid, malonate, acetate, lactic acid, L-alanine, 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, L-arabinose, propionic acid, valeric acid, L-histidine, 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, and L-proline.

Characteristics	1	2	3	4	5	6
Oxidase	+	-[a]	-[b]	+[c]	+[d]	+[e]
Colony color[†]	DY-O	O[a]	Y-LO[b]	DY[c]	O[d]	O[e]
Highest NaCl concentration tolerated (%)	3.0	3.0[a]	2.0[b]	1.0[c]	1.0[d]	1.0[e]
Indole production	+	+	-	+	+	+
Hydrolysis of						
CM-cellulose	+	-	-	-	-	+
Gelatin	+	+	-	-	+	+
Assimilation of						
D-Mannitol	+	-	-	+	-	-
D-Ribose	-	-	-	+	+	+
Inositol	-	+	-	-	-	-
L-Fucose	+	-	-	-	-	+
D-Sorbitol	+	-	-	-	-	-
Enzyme activity						
Valine arylamidase	+	-	-	+	-	-
Trypsin	+	-	+	-	-	+
β-Glucosidase	-	+	-	+	+	-
DNA G+C content (mol%)	43.6	45[a]	43[b]	45[c]	46.9[d]	42[e]

[a] Data are taken from Kim *et al.* (2007).
[b] Data are taken from Weon *et al.* (2009).
[c] Data are taken from, Weon *et al.* (2008).
[d] Data are taken from Dai *et al.* (2011).
[e] Data are taken from Wang *et al.* (2009).
[†] DY, Dark yellow; O, orange; Y, yellow; LO, light orange.

Table 2. Cellular fatty acid composition of strain ICM 1-15^T and type strains of *Niabella* species
Strains: 1, ICM 1-15^T; 2, *N. aurantiaca* KACC 11698^T; 3, *N. ginsengisoli* KACC 13021^T; 4, *N. soli* KACC 12604^T; 5, *N. tibetensis* KACC 15620^T; 6, *N. yanshanensis* KACC 14980^T. All data are from this study. Only fatty acids that represent more than 1% of the total fatty acids are indicated. All strains were grown on R2A agar at 28°C for 3 days. Values are percentages of total fatty acids. -, Not detected.

Fatty acid	1	2	3	4	5	6
anteiso-$C_{15:0}$	3.8	1.2	-	1.2	1.0	-
iso-$C_{15:0}$	38.9	30.4	30.6	43.2	40.0	34.3
iso-$C_{15:0}$ 3-OH	3.0	2.6	3.2	3.0	2.6	3.0
iso-$C_{15:1}$ G	20.3	21.6	30.2	18.5	21.2	26.7
$C_{16:0}$	2.6	5.7	3.9	4.8	3.9	3.5
$C_{16:0}$ 3-OH	1.8	2.4	2.6	1.5	2.7	1.6
iso-$C_{16:0}$	2.5	-	-	-	-	-
iso-$C_{16:0}$ 3-OH	2.2	-	-	-	-	-
$C_{16:1}\omega5c$	-	-	1.8	-	-	1.8
iso-$C_{17:0}$ 3-OH	12.9	14.1	12.4	12.5	13.5	15.4
$C_{17:1}\omega6c$	1.8	-	-	-	-	-
$C_{18:1}\omega9c$	-	-	-	1.3	-	-
Summed feature 3[a]	5.3	10.3	11.6	9.3	11.3	8.7
Summed feature 8[a]	-	2.1	-	-	-	-

[a] Summed features are groups of two or three fatty acids that cannot be separated by the MIDI system. Summed feature 3 comprised $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; summed feature 8 comprised $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

L-fucose was assimilated only by strain ICM 1-15^T and *N. yanshanensis* KACC 14980^T. D-sorbitol was assimilated only by strain ICM 1-15^T among the type strains of the genus *Niabella*. Valine arylamidase activity was observed only by strain ICM 1-15^T and *N. soli* KACC 12604^T.

Chemotaxonomy

Similar to other type strains of the genus *Niabella* (Dai *et al.*, 2011), menaquinone-7 (MK-7) was the major isoprenoid quinone, and iso-$C_{15:0}$ (38.9%), iso-$C_{15:1}$ G (20.3%), and iso-$C_{17:0}$ 3-OH (12.9%) were the major fatty acids (>10%) in strain ICM 1-15^T. The complete fatty acid composition is provided in Table 2. The fatty acids iso-$C_{16:0}$, iso-$C_{16:0}$ 3-OH, and $C_{17:1}\omega6c$ were observed only in this strain, which also had a relatively higher amount of anteiso-$C_{15:0}$ and lower amounts of $C_{16:0}$ and summed feature 3 than did other type strains of the genus *Niabella*.

Taxonomic conclusion

In conclusion, strain ICM 1-15^T is similar to other type strains of the genus *Niabella,* because it produces flexirubin pigment and has iso-$C_{15:0}$, iso-$C_{15:1}$ G, and iso-$C_{17:0}$ 3-OH as the major fatty acids, but it can be distinguished from the other type strains by its low 16S rRNA gene sequence similarity and based on distinct phenotypic characteristics such as the assimilation of D-sorbitol and the presence of iso-$C_{16:0}$, iso-$C_{16:0}$ 3-OH, and $C_{17:1}\omega6c$ as membrane fatty acids. Thus, strain ICM 1-15^T represents a novel species in the genus *Niabella*, for which the name *Niabella terrae* sp. nov. is proposed.

Description of *Niabella terrae* sp. nov.

Niabella terrae (ter´rae. L. gen. n. *terrae* of the soil).
Cells are strictly aerobic, Gram-staining-negative, non-spore-forming, non-motile rods, 0.4-0.6 μm wide, and 0.6–1.1 μm long. Grows at 15–35°C (optimum, 25°C), at pH 5.0–8.5 (optimum, pH 6.5) and in the presence of 0–3% NaCl (optimum, 1%). Produces flexirubin pigments. Colonies on R2A medium are dark yellow to orange, circular, convex with entire margins. Growth occurs on TSA, NA, LB, and MA media. Positive for catalase and oxidase. Starch and CM-cellulose are hydrolysed. Casein, lipid, and chitin are not hydrolysed. Positive for indole production, esculin hydrolysis, and gelatin hydrolysis, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, and urease (API 20NE). Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities, but negative for lipase (C14), crystine arylamidase, α-chymotrypsin, β-glucuronidase, and β-glucosidase activities (API ZYM). Assimilates D-glucose, L-arabinose, D-mannose, D-manitol, *N*-acetyl-glucosamine and not assimilates gluconate, capric acid, adipic acid, malic acid, citrate, and phenylacetic acid (API 20NE). Assimilates L-rhamnose, D-saccharose, D-maltose, glycogen, salicin, D-melibiose, L-fucose, and D-sorbitol and not assimilates D-ribose, inositol, itaconic acid, suberic acid, malonate, acetate, lactic acid, L-alanine, 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, propionic acid, valeric acid, L-histidine, 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, and L-proline (API ID 32 GN). The major isoprenoid quinone is MK-7. The predominant fatty acids (>10%) are iso-$C_{15:0}$, iso-$C_{15:1}$ G, and iso-$C_{17:0}$ 3-OH. The DNA G+C content of the type strain is 43.6 mol%. The type strain, ICM 1-15^T (=KACC 17443^T =JCM 19502^T), was isolated from a greenhouse soil cultivated with lettuce in Incheon, Korea.

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