Niabella tibetensis sp. nov., isolated from soil, and emended description of the genus *Niabella*

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Strain 15-4^T, a Gram-stain-negative, rod-shaped, non-motile bacterial strain that produced flexirubin-type pigments, was isolated from Tibet Province, China, and characterized by using a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate belonged to the family *Chitinophagaceae*, phylum 'Bacteroidetes', and was related to members of the genus *Niabella*, with sequence similarities ranging from 94.1 to 96.4 %. Strain 15-4^T contained MK-7 as the predominant menaquinone and its DNA G + C content was 46.9 mol%. The major fatty acids of strain 15-4^T were iso-C_{15:0} (41.3 %), iso-C_{15:1} G (14.9 %), iso-C_{17:0} 3-OH (13.2 %) and summed feature 3 (comprising C_{16:1ω7c} and/or iso-C_{15:0} 2-OH, 16.0 %). These chemotaxonomic data supported the affiliation of strain 15-4^T to the genus *Niabella*. However, a number of physiological and biochemical features enabled the isolate to be differentiated phenotypically from recognized species of the genus *Niabella*. On the basis of the evidence presented, it is proposed that strain 15-4^T represents a novel species, *Niabella tibetensis* sp. nov.; the type strain is 15-4^T (=CCTCC AB 209167^T=NRRL B-59394^T). On the basis of these data, an emended description of the genus *Niabella* is also proposed.

The genus *Niabella* was first described by Kim *et al.* (2007) to accommodate Gram-stain-negative, aerobic, non-flagellated and non-gliding, short rod-shaped bacteria that produce flexirubin-type pigments. The genus *Niabella* belongs to the family *Chitinophagaceae*, phylum 'Bacteroidetes' (Ludwig *et al.*, 2008), and, at the time of writing, comprised four recognized species, all isolated from soil: *Niabella aurantiaca* (Kim *et al.*, 2007), *N. soli* (Weon *et al.*, 2008), *N. yanshanensis* (Wang *et al.*, 2009) and *N. ginsengisoli* (Weon *et al.*, 2009). During the course of an investigation of the culturable bacterial community in soil from Tibet Province, China, a large number of bacteria were isolated. In this study, the taxonomic characterization of one of these isolates, designated strain 15-4^T, is reported. On the basis of phenotypic data and phylogenetic inference, the isolate represents a novel species of the genus *Niabella*.

Strain 15-4^T was isolated from a sample taken from the top 20 cm layer of soil collected from Xietongmen district (88° 15' E 29° 25' N). The soil sample was thoroughly suspended in sterilized water and the suspension was spread on R2A agar (Difco) after serial dilution with sterilized water. Isolation was achieved after incubation at 28 °C for 1 week. Strain 15-4^T was cultivated routinely on

the same medium at 30 °C and preserved at –80 °C as a suspension in distilled water containing 20 % (v/v) glycerol.

Cell morphology was examined by using phase-contrast microscopy (BX51; Olympus). Gliding motility was examined as described by Bowman (2000) and Gram staining was performed as described by Gerhardt *et al.* (1994). In addition to R2A agar, growth of strain 15-4^T was tested on trypticase soy broth (TSB; Difco), nutrient agar (NA; Difco) and marine agar 2216 (MA; Difco). Growth conditions were determined by investigating the growth of strain 15-4^T at various pH (pH 4–10, at unit intervals), temperatures (4, 10, 15, 20, 30, 34 and 37 °C) and NaCl concentrations [0, 1, 2, 3 and 5 % (w/v)] on R2A agar for up to 1 week. The pH was adjusted to between pH 4.0 and 9.0 with stock solutions of NaHCO₃ (10 %, w/v), Na₂CO₃ (10 %, w/v) and citrate buffer (1 M), and to pH 10.0 with the buffer system of Na₂HPO₄ (0.05 M) and NaOH (0.1 M). Catalase activity was determined using a 3 % (v/v) hydrogen peroxide solution. Oxidation of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine was used to determine oxidase activity. Growth under anaerobic conditions was tested on R2A agar in a GasPak (BBL) jar at 30 °C for 10 days. In order to compare the phenotypic characteristics of strain 15-4^T with those of the other species of the genus *Niabella*, *N. aurantiaca* KACC 11698^T, *N. soli* KACC 12604^T and *N. ginsengisoli* GR10-1^T (obtained from the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 15-4^T is GU291295.

Korean Agricultural Culture Collection, Suwon, Korea) and *N. yanshanensis* CCBAU 05354^T (obtained from the China Center for Type Culture Collection, Wuhan, PR China) were used as reference strains. The following tests were performed on the five strains. Hydrolysis of carb-oxy-methylcellulose, casein, chitin and starch was determined using the methods of Smibert & Krieg (1994). Flexirubin-type pigments were detected by means of a colour shift after exposure of colonies to 20 % (w/v) KOH solution (Reichenbach, 1992). Other biochemical tests were carried out by using API 20NE, API ID 32GN and API ZYM test kits according to the manufacturer's instructions (bioMérieux). The phenotypic features of strain 15-4^T are given in the species description and in Table 1.

Chromosomal DNA was isolated and purified by using a Bacteria Genomic DNA isolation kit (ChaoShi-Bio). The 16S rRNA gene was amplified by PCR with universal bacterial primers 27F and 1527R, which were also used for sequencing (Lane, 1991). An almost-complete 16S rRNA gene sequence of strain 15-4^T was obtained and compared with sequences deposited in GenBank. Sequences were aligned using the CLUSTAL_X software (Thompson *et al.*, 1997) and distances were calculated according to Kimura's two-parameter method (Kimura, 1980). Phylogenetic trees were constructed using three tree-making algorithms,

i.e. the neighbour-joining, minimum-evolution and maximum-parsimony methods, using MEGA version 3.1 (Kumar *et al.*, 2004).

Analysis of the 16S rRNA gene sequence of strain 15-4^T resulted in a sequence of 1409 bp. Sequence similarity values to recognized members of the genus *Niabella* were below the threshold for demarcating bacterial species (Stackebrandt & Goebel, 1994), as follows: *N. aurantiaca* KACC 11698^T (96.4 %), *N. soli* KACC 12604^T (95.3 %), *N. yanshanensis* CCBAU 05354^T (94.8 %) and *N. ginsengisoli* GR10-1^T (94.1 %). No other recognized bacterial species showed more than 95 % 16S rRNA gene sequence similarity to the novel isolate. In the phylogenetic tree based on the neighbour-joining algorithm, strain 15-4^T was clearly placed within the genus *Niabella*, forming a separate branch with *N. aurantiaca* KACC 11698^T (Fig. 1). The maximum-parsimony and minimum-evolution phylogenetic trees showed essentially the same topology (data not shown). These results suggest that strain 15-4^T represents a novel species within the genus *Niabella*.

The DNA G + C content of strain 15-4^T was determined by using HPLC according to the method of Mesbah *et al.* (1989). Isoprenoid quinones were extracted and identified by HPLC as described by Xie & Yokota (2003). To determine the whole-cell fatty acid profile, strain 15-4^T and

Table 1. Differential characteristics of strain 15-4^T and other species of the genus *Niabella*

Strains: 1, 15-4^T; 2, *N. aurantiaca* KACC 11698^T; 3, *N. soli* KACC 12604^T; 4, *N. ginsengisoli* GR10-1^T; 5, *N. yanshanensis* CCBAU 05354^T. All strains are positive for production of flexirubin-type pigments, assimilation of D-glucose, L-arabinose, D-mannose, N-acetylglucosamine, maltose, L-rhamnose, sucrose, salicin and melibiose, aesculin hydrolysis and β -galactosidase activity. All strains are negative for gliding motility, nitrate reduction, glucose acidification, arginine dihydrolase and urease. All data are from this study except the DNA G + C content of the four reference strains [taken from Kim *et al.* (2007), Weon *et al.* (2008, 2009) and Wang *et al.* (2009)]. +, Positive; –, negative; (+), weakly positive.

Characteristic	1	2	3	4	5
Highest NaCl concentration tolerated (%)	1.0	3.0	1.0	2.0	1.0
Catalase/oxidase activities	+/+	+/-	-/+	+/-	+/+
Casein hydrolysis	+	+	–	–	+
Gelatin hydrolysis	–	–	–	–	+
Indole production	+	+	+	–	+
Assimilation of (API ID 32GN):					
Glycogen	–	–	–	–	+
Malic acid	–	–	–	–	+
D-Mannitol	–	–	+	–	+
Phenylacetic acid	–	–	–	–	+
Potassium gluconate	–	–	–	–	+
D-Ribose	+	–	+	+	+
Trisodium citrate	–	–	–	–	+
Enzyme activity (API ZYM):					
Cystine arylamidase	(+)	–	(+)	–	+
α -Mannosidase	(+)	–	–	(+)	+
Trypsin	–	–	–	–	+
Valine arylamidase	–	–	–	–	+
DNA G + C content (mol%)	46.9	45	45	43	42

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain 15-4^T and representative members of the family Chitinophagaceae. Only bootstrap values (percentages of 1000 replications) ≥ 70 % are shown at nodes. *Balneola vulgaris* 13IX/A01/164^T and *Gracilimonas tropica* CL-CB462^T were used as the outgroup. Bar, 0.05 substitutions per nucleotide position.

the four reference strains were grown on R2A agar at 30 °C for 48 h. Analysis of fatty acid methyl esters was carried out using GC (6890; Hewlett Packard) according to the instructions of the Sherlock Microbial Identification System (MIDI).

The DNA G + C content of strain 15-4^T was 46.9 mol%, a value that was slightly higher than those reported for the other species of the genus *Niabella* (Table 1). The predominant isoprenoid quinone was MK-7, which is in line with that found in all members of the family Chitinophagaceae. The major fatty acids of strain 15-4^T were iso-C_{15:0} (41.3 %), iso-C_{15:1} G (14.9 %), iso-C_{17:0}

3-OH (13.2 %) and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, 16.0 %). As shown in Table 2, the fatty acid compositions of strain 15-4^T, *N. aurantiaca* KACC 11698^T, *N. soli* KACC 12604^T, *N. yanshanensis* CCBAU 05354^T and *N. ginsengisoli* GR10-1^T were very similar, with only minor differences in the respective proportions of some components.

Based on phylogenetic inference and phenotypic data, strain 15-4^T represents a novel species of the genus *Niabella*, for which the name *Niabella tibetensis* sp. nov. is proposed. As a consequence of newly reported characteristics, an emended description of the genus *Niabella* is given.

Table 2. Fatty acid profile of strain 15-4^T and other members of the genus *Niabella*

Strains: 1, 15-4^T; 2, *N. aurantiaca* KACC 11698^T; 3, *N. soli* KACC 12604^T; 4, *N. ginsengisoli* GR10-1^T; 5, *N. yanshanensis* CCBau 05354^T. Values are percentages of total fatty acids. All data are from this study. Fatty acids amounting to <0.5 % of the total fatty acids in all strains listed are not shown. tr, Trace (<0.5 %); ND, not detected.

Fatty acid	1	2	3	4	5
C _{14:0}	0.8	0.6	0.6	1.1	1.3
iso-C _{15:1} G	14.9	17.7	13.0	23.8	24.5
iso-C _{15:0}	41.3	34.4	44.2	35.2	34.8
anteiso-C _{15:0}	0.9	1.3	1.3	0.5	tr
iso-C _{16:0}	tr	tr	ND	1.3	0.6
C _{16:0}	3.4	3.6	3.4	3.7	4.1
iso-C _{15:0} 3-OH	2.4	2.4	2.1	3.8	3.1
C _{15:0} 2-OH	0.5	2.5	1.4	ND	tr
iso-C _{16:0} 3-OH	0.7	ND	ND	0.9	0.5
C _{15:0} 3-OH	ND	0.5	ND	ND	ND
C _{16:0} 2-OH	ND	0.7	ND	0.5	tr
C _{16:0} 3-OH	3.3	3.0	2.0	2.9	1.7
iso-C _{17:0} 3-OH	13.2	15.2	13.9	12.6	14.2
C _{17:0} 2-OH	tr	0.6	0.5	ND	ND
C _{17:0} 3-OH	ND	1.2	0.6	ND	ND
Summed feature 3*	16.0	14.1	15.0	10.2	10.2
Unknown 13.565†	0.9	0.9	1.0	0.6	0.5
Unknown 16.582†	1.7	1.4	1.2	0.9	1.2

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified; values are equivalent chain-lengths.

Emended description of the genus *Niabella* Kim *et al.* 2007

This emended description takes into account results from Kim *et al.* (2007), Weon *et al.* (2008, 2009) and Wang *et al.* (2009), together with those from this study.

Cells are Gram-stain-negative, strictly aerobic, non-flagellated, non-gliding, non-spore-forming, short rods. Flexirubin-type pigments are produced. Catalase and oxidase activities are species-dependent. The predominant isoprenoid quinone is MK-7. The major fatty acids are iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c). The DNA G+C contents are approximately 42–47 mol%. Phylogenetically, the genus is a member of the phylum 'Bacteroidetes'. The type species is *Niabella aurantiaca*.

Description of *Niabella tibetensis* sp. nov.

Niabella tibetensis (ti.be.ten'sis. N.L. fem. adj. *tibetensis* of or belonging to Tibet, an autonomous region in north-west China).

Cells are strictly aerobic, short rods, 0.5–0.8 µm in diameter and 1.1–1.7 µm in length, which are non-flagellated, non-gliding and Gram-stain-negative. Growth occurs on R2A agar after incubation at 30 °C for 48 h. Good growth also occurs on TSA and NA, but not on MA. Colonies on R2A agar are orange, convex and circular, with regular margins. Growth occurs with 0–1 % NaCl (w/v), at 15–34 °C (optimum, 25–30 °C) and at pH 5.0–8.0 (optimum, pH 6.0–7.0). Produces flexirubin-type pigments. Catalase and oxidase activities are present. Hydrolyses casein, but not chitin, carboxymethylcellulose, DNA or starch. Positive for indole production, aesculin hydrolysis and β-galactosidase activity, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis (API 20NE). Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are present, but lipase (C14), valine arylamidase, trypsin, α-chymotrypsin and β-glucuronidase activities are absent (API ZYM). D-Glucose, L-arabinose, D-mannose, N-acetylglucosamine, maltose, L-rhamnose, D-ribose, sucrose, salicin and melibiose are assimilated (API 20NE and API ID 32GN). D-Mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline are not assimilated. The predominant isoprenoid quinone is MK-7. The major cellular fatty acids (>13 %) are iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH).

The type strain is 15-4^T (=CCTCC AB 209167^T=NRRL B-59394^T), isolated from the surface layer of a desert soil from Tibet Province, China. The DNA G+C content of the type strain is 46.9 mol%.

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