Niabella ginsengisoli sp. nov., isolated from soil cultivated with Korean ginseng

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The taxonomic status of a yellow- to light orange-coloured strain isolated from soil of a Korean ginseng field was established based on a polyphasic investigation. The novel isolate, strain GR10-1^T, was an obligately aerobic, Gram-staining-negative, non-motile, flexirubin-pigment-producing, short rod-shaped bacterium. The strain grew optimally at 28–30 °C, at pH 7.0 and in the presence of 0–1 % NaCl. Phylogenetic analyses based on 16S rRNA gene sequences demonstrated that the new isolate showed the highest sequence similarities with *Niabella aurantiaca* R2A15-11^T (95.1 %) and *Niabella soli* JS13-8^T (94.6 %). The DNA G+C content of strain GR10-1^T was 43 mol%. It contained iso-C_{15:1} G (36.4 %) and iso-C_{15:0} (32.8 %) as the major fatty acids (>10 %) and MK-7 as the major isoprenoid quinone. On the basis of evidence from our polyphasic taxonomic study, it was concluded that strain GR10-1^T should be classified within a novel species of the genus *Niabella*, for which the name *Niabella ginsengisoli* sp. nov. is proposed. The type strain is GR10-1^T (=KACC 13021^T =JCM 15444^T).

The genus *Niabella* was proposed by Kim *et al.* (2007) for a bacterium isolated from soil, *Niabella aurantiaca*. *Niabella soli*, also from soil (Weon *et al.*, 2008), was described subsequently. The genus comprises strictly aerobic, Gram-negative, non-flagellated, flexirubin-pigment-producing, short rods. Phylogenetically, the genus is a member of the phylum *Bacteroidetes*, closely related to the genera *Terrimonas* (Xie & Yokota, 2006) and *Niastella* (Weon *et al.*, 2006).

In the course of the study of bacterial diversity in fields cultivated with Korean ginseng (*Panax ginseng* Meyer), a novel bacterial strain was isolated in Yeongju region. For isolation, the soil sample was diluted serially in saline solution (0.85 % w/v), spread on R2A agar (Reasoner & Geldreich, 1985) and incubated for 5 days at 30 °C.

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 analysed using an Applied Biosystems ABI3100 DNA sequencer. The nearly complete 16S rRNA gene sequence

of strain GR10-1^T (1459 nt) was obtained and aligned with sequences retrieved from GenBank using the CLUSTAL W software (Thompson *et al.*, 1994). Nucleotide similarity values were calculated using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic trees for the datasets were inferred from the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using MEGA version 3.0 (Kumar *et al.*, 2004). The stability of relationships was assessed by performing bootstrap analyses based on 1000 resamplings.

Analyses of the 16S rRNA gene sequences showed that strain GR10-1^T shared the highest sequence similarities with *Niabella aurantiaca* R2A15-11^T (95.1 %) and *Niabella soli* JS13-8^T (94.6 %), and <94 % sequence similarity with the other strains included in the analysis. The maximum-parsimony tree indicated that strain GR10-1^T formed a clade with *Niabella aurantiaca* and *Niabella soli* with a 99 % bootstrap value (Fig. 1). The next most closely related neighbours of strain GR10-1^T were members of the genus *Terrimonas*. The topology of the neighbour-joining tree was exactly the same (not shown).

Phenotypic characterization was carried out as described previously (Kim *et al.*, 2007) and according to standard methods (Smibert & Krieg, 1994), using R2A medium as the basal medium at 30 °C. For transmission electron microscopy, cells were grown for 24 h, negatively stained with 0.5 % (w/v) uranyl acetate and examined with a LEO

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GR10-1^T is EU616816.

A table listing additional differentiating phenotypic characteristics between *Niabella* species and an electron micrograph of cells of strain GR10-1^T are available as supplementary material with the online version of this paper.

Fig. 1. Maximum-parsimony tree based on a comparative analysis of 16S rRNA gene sequences showing the relationships of strain GR10-1^T with related taxa. Bootstrap values (expressed as percentages of 1000 replications) are shown at branching points. Bar, 50 expected changes.

model 912AB electron microscope. Growth under anaerobic conditions was tested in a GasPak (BBL) jar at 30 °C for 15 days on R2A medium. Flexirubin-type pigments were revealed by the colour 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 ID 32GN and API ZYM systems, following the instructions of the manufacturer (bioMérieux).

Cells of strain GR10-1^T were strictly aerobic, Gram-negative rods that exhibited pili-like structures but no flagella (see Supplementary Fig. S1, available in IJSEM

Online). On R2A medium, colonies were yellow at an early stage, but they turned to a light-orange colour after 1 week. Other phenotypic characteristics of strain GR10-1^T and closely related species are shown in Table 1.

The presence of isoprenoid quinones was investigated using HPLC, as described previously (Groth *et al.*, 1996). Fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification System (MIDI; Microbial ID) from cells grown on R2A for 48 h at 30 °C. The DNA G + C content was determined according to Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18S; Supelco).

Table 1. Differential characteristics of strain GR10-1^T and type strains of *Niabella* species and related species

Strains: 1, GR10-1^T; 2, *Niabella aurantiaca* KACC 11698^T (data from Kim *et al.*, 2007); 3, *Niabella soli* KACC 12604^T (Weon *et al.*, 2008); 4, *Terrimonas ferruginea* IAM 15098^T (data from Xie & Yokota, 2006 unless indicated); 5, *Terrimonas lutea* IAM 15284^T (Xie & Yokota, 2006); 6, *Niastella koreensis* KACC 11465^T (Weon *et al.*, 2006); 7, *Niastella yeongjuensis* KACC 11466^T (Weon *et al.*, 2006). +, Positive; (+), weakly positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Catalase/oxidase	+/-	+/-	+/+	(+)/+	(+)/+	-/-	-/+
Flexirubin pigments	+	+	+	—*	ND	—	—
Gliding motility	—	—	—	—	—	+	+
Colony colour†	Y-LO	O	DY	SR	Y	LY	MW
Nitrate reduction	—	—	—	+	+	—	—
Indole production	—	+	—	—	—	—	—
Highest NaCl concentration tolerated (%)	2.0	3.0	1.0	1.0	1.0	ND	ND
Hydrolysis of:							
Casein	—	+	—	—*	ND	+	+
Chitin	—	—	—	—	—	+	+
CM-cellulose	—	—	—	—*	ND	+	+
Gelatin	—	+	—	+	+	+	+
Starch	+	—	—	+	ND	—	—
DNA G + C content (mol%)	43	45	45	48.9	47.2	45.8	44.3

*Data from Weon *et al.* (2006).

†DY, Dark yellow; LO, light orange; LY, light yellow; MW, milky white; O, orange; SR, salmon red; Y, yellow.

Strain GR10-1^T had menaquinone 7 (MK-7) as the major isoprenoid quinone and iso-C_{15:1} G (36.4 %) and iso-C_{15:0} (32.8 %) as the major fatty acids (>10 %). The complete fatty acid composition is given in Table 2. The DNA G + C content of strain GR10-1^T was 43 mol%.

Strain GR10-1^T displayed key characteristics of the genus *Niabella* such as the ability to produce flexirubin-type pigments and catalase and the lack of gliding motility and nitrate reduction, in contrast to members of the genera *Terrimonas* and *Niastella*. In addition to differences in its fatty acid composition, substrate assimilation and enzyme profile, strain GR10-1^T could be differentiated from *Niabella aurantiaca* by the lack of indole production, the inability to hydrolyse casein and gelatin and the ability to hydrolyse starch and from *Niabella soli* by the absence of oxidase activity and the ability to hydrolyse starch (Tables 1 and 2 and Supplementary Table S1).

On the basis of these results, strain GR10-1^T represents a novel species of the genus *Niabella*, for which the name *Niabella ginsengisoli* sp. nov. is proposed.

Description of *Niabella ginsengisoli* sp. nov.

Niabella ginsengisoli (gin.seng.i.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil from a ginseng field, the source of the type strain).

Cells are strictly aerobic, Gram-staining-negative, non-motile rods, 0.6–0.8 µm wide and 1.2–1.7 µm long, and exhibit pili-like structures. Grows at 5–35 °C (optimum, 28–30 °C), at pH 6.0–8.0 (optimum, pH 7.0) and in the presence of 0–2 % NaCl (optimum, 0–1 %). Colonies on R2A medium are yellow to light orange, circular and convex with entire margins. The non-diffusible pigments belong to the flexirubin type. Positive for catalase activity and aesculin hydrolysis. Negative for oxidase, arginine

Table 2. Cellular fatty acid compositions of strain GR10-1^T and type strains of related species

Strains: 1, GR10-1^T; 2, *Niabella aurantiaca* KACC 11698^T (Kim *et al.*, 2007); 3, *Niabella soli* KACC 12604^T (Weon *et al.*, 2008); 4, *Terrimonas ferruginea* IAM 15098^T (Xie & Yokota, 2006); 5, *Terrimonas lutea* IAM 15284^T (Xie & Yokota, 2006); 6, *Niastella koreensis* KACC 11465^T (Weon *et al.*, 2006); 7, *Niastella yeongjuensis* KACC 11466^T (Weon *et al.*, 2006). Strains GR10-1^T, KACC 11698^T, KACC 12604^T, KACC 11465^T and KACC 11466^T were grown on R2A agar for 48 h at 28–30 °C. Strains IAM 15098^T and IAM 15284^T were grown on trypticase soy agar (Becton Dickinson) for 3 days at 29 °C. Only fatty acids that represent more than 1 % of the total fatty acids are indicated; –, <1 % or not reported.

Fatty acid	1	2	3	4	5	6	7
iso-C _{14:0}	–	–	–	1.1	4.3	–	–
C _{15:0}	–	–	–	2.7	–	–	–
anteiso-C _{15:0}	–	1.6	1.2	–	–	4.9	3.3
iso-C _{15:0}	32.8	33.7	29.2	28.4	34.8	26.8	30.6
iso-C _{15:0} 3-OH	3.7	2.9	2.2	2.2	2.3	1.3	1.9
iso-C _{15:1} G	36.4	22.3	18.4	26.2	21.8	15.6	14.7
C _{16:0}	7.9	3.5	6.8	1.7	1.1	2.6	2.9
C _{16:0} 2-OH	–	–	1.3	–	–	–	–
C _{16:0} 3-OH	–	2.4	2.2	2.5	3.3	1.3	2.3
iso-C _{16:0}	–	–	–	1.4	–	1.0	–
C _{16:1} ω5c	–	–	–	–	–	–	1.2
C _{17:0} 2-OH	–	–	–	–	–	3.5	2.6
C _{17:0} 3-OH	–	–	–	–	–	1.2	–
iso-C _{17:0}	–	–	–	–	–	1.6	–
iso-C _{17:0} 3-OH	9.5	15.5	11.8	15.3	14.2	29.4	27.1
C _{18:0}	–	–	3.8	–	–	–	–
C _{18:1} ω7c	–	–	1.5	–	–	–	–
C _{18:1} ω9c	–	–	1.0	–	–	–	–
Summed feature 3*	3.5	10.6	11.1	11.2	14.9	4.3	5.3
Summed feature 5*	–	–	3.4	–	–	–	–
Unknown 13.565	6.1	–	1.1	1.3	3.2	–	–
Unknown 16.582	–	1.2	–	1.3	1.8	1.4	1.6

*Summed features are groups of two or three fatty acids that cannot be separated by the MIDI system. Summed feature 3 comprised C_{15:0} iso 2-OH and/or C_{16:1}ω7c; summed feature 5 comprised anteiso-C_{18:0} and/or C_{18:2}ω6,9c.

dihydrolase and urease activities, nitrate reduction and glucose fermentation. Hydrolyses starch and tyrosine, but not alginic acid, casein, chitin, CM-cellulose or DNA. The assimilation profile and enzyme activities are shown in Supplementary Table S1 and other phenotypic characteristics are shown in Table 1. The major isoprenoid quinone is MK-7. The predominant fatty acids (>5%) are iso-C_{15:1} G, iso-C_{15:0}, iso-C_{17:0} 3-OH, C_{16:0} and unknown 13.565. The DNA G+C content of the type strain is 43 mol%.

The type strain, GR10-1^T (=KACC 13021^T =JCM 15444^T), was isolated from soil cultivated with Korean ginseng (*Panax ginseng* Meyer) in Yeongju region, Republic of Korea.

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