Niabella yanshanensis sp. nov., isolated from the soybean rhizosphere

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An orange-coloured bacterium, CCBAU 05354^T, was isolated from the soybean rhizosphere following growth on NA medium. The sample was taken from a field in Hebei province, People's Republic of China. The cells were aerobic, Gram-stain-negative, short rods (0.4–0.6×0.7–1.7 μm) and non-motile. Growth occurred at 28 °C (not at 10 or 37 °C), pH 6.0–10.0, and in the presence of 0–1 % NaCl (w/v). Flexirubin pigment was produced and the cells were resistant to some antibiotics. A phylogenetic analysis based on 16S rRNA gene sequences indicated that strain CCBAU 05354^T was closely related to *Niabella soli* KACC 12604^T (95.1 % sequence similarity). The predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The DNA G+C content was 42 mol%. On the basis of the phylogenetic, phenotypic and chemotaxonomic data, strain CCBAU 05354^T represents a novel species of the genus *Niabella*, for which the name *Niabella yanshanensis* sp. nov. is proposed. The type strain is CCBAU 05354^T (=LMG 24661^T =HAMBI 3031^T).

The genus *Niabella* and the type species of the genus, *Niabella aurantiaca*, were proposed by Kim *et al.* (2007), and another species, *Niabella soli*, was proposed by Weon *et al.* (2008). Cells of this genus were characterized as Gram-negative, aerobic, non-flagellated, short rods and flexirubin-pigment-producing. Colonies are orange and circular on R2A plates. 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 $C_{16:1}\omega7c$).

In the course of a study on the bacterial diversity of the soybean rhizosphere from Hebei, People's Republic of China, we isolated an orange-coloured bacterial strain. The soil sample was serially diluted with 0.85 % NaCl (w/v) and the dilutions were plated onto nutrient agar (NA) agar. The strain was isolated after incubation for 4 days at 28 °C.

Phenotypic characteristics, including Gram-staining, catalase activity, oxidase activity, nitrate reduction, glucose fermentation, urease activity and hydrolysis of CM-cellulose, DNA, Tween 80, starch, phosphatidylcholine and gelatin, were evaluated using the methods of Smibert & Krieg (1994). Cell morphology, motility and flagellation were investigated using phase-contrast and transmission electron microscopy with cells that had been negatively stained with 0.5 % uranyl acetate. Growth at different temperatures was tested at 4, 10, 28, 37, 45 and 60 °C, and growth at varying pH was tested at pH 5–12 (in increments of 1 pH unit). Salt tolerance was tested in nutrient broth supplemented with 0, 1, 2, 3, 4 and 5 % (w/v) NaCl. Antibiotic resistance was tested by growth on NA agar supplemented with ampicillin, gentamicin, kanamycin, chloramphenicol, neomycin and erythromycin at concentrations of 5, 50, 100 and 300 μg ml^{-1}. Flexirubin pigment was detected as a colour shift after exposure to a 20 % (w/v) KOH solution (Reichenbach, 1992). Other metabolic fingerprinting was carried out by using a Biolog GN2 MicroPlate according to the manufacturer's instructions. Phenotypic comparisons between strain CCBAU 05354^T, *N. soli* KACC 12604^T (=JS13-8^T) and *N. aurantiaca* KACC 11698^T (=R2A15-11^T) are shown in Table 1.

The cellular fatty acids of strain CCBAU 05354^T were analysed using cells grown on R2A agar for 2 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of Sasser (1990). The G+C

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of CCBAU 05354^T is FJ457040.

Table 1. Phenotypic characteristics that differentiate strain CCBAU 05354^T from *N. soli* KACC 12604^T and *N. aurantiaca* KACC 11698^T

Strains: 1, *N. yanshanensis* sp. nov. CCBAU 05354^T; 2, *N. soli* KACC 12604^T; 3, *N. aurantiaca* KACC 11698^T. Data for *N. soli* KACC 12604^T were taken from Weon *et al.* (2008) with the exception of the antibiotic resistance (from this study). Data for *N. aurantiaca* KACC 11698^T were taken from Kim *et al.* (2007). +, Positive; −, negative; ND, no data available. Only fatty acids that account for more than 1 % of the total are indicated.

Characteristic	1	2	3
Tween 80 hydrolysis	−	+	ND
Starch hydrolysis	+	−	−
Glucose fermentation	+	−	−
Assimilation of:			
Glycogen	+	−	−
L-Fucose	+	−	−
DL-Lactic acid	+	−	−
pH range for growth	6.0–10.0	5.0–8.0	5.0–8.0
Antibiotic resistance (μg ml^{-1})			
Ampicillin	5	−	ND
Gentamicin	5	−-	ND
Kanamycin	5	−	ND
Chloramphenicol	5	−	ND
Neomycin	50	−	ND
Erythromycin	100	−	ND
DNA G+C content (mol%)	42	45	45
Fatty acids (% of total)			
iso-$C_{14:0}$	1.0	−	−
$C_{14:0}$	1.1	−	−
iso-$C_{15:1}$ G	30.5	18.4	22.3
iso-$C_{15:0}$	39.4	29.2	33.7
$C_{16:1}\omega5c$	1.1	−	−
$C_{16:0}$	4.3	6.8	3.5
iso-$C_{15:0}$ 3-OH	3.2	2.2	2.9
$C_{16:0}$ 3-OH	1.4	2.2	2.4
iso-$C_{17:0}$ 3-OH	7.7	11.8	15.5
Summed feature 3*	7.8[a]	11.1[b]	10.6[b]

*Summed feature 3 comprises a, $C_{16:0}\omega7c$ and/or $C_{16:0}\omega6c$; b, iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

content of the chromosomal DNA was analysed as described by De Ley (1970) and was found to be 42 mol%. The major cellular fatty acids were iso-$C_{15:0}$ (39.4 %), iso-$C_{15:1}$ G (30.5 %), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 7.8 %) and iso-$C_{17:0}$ 3-OH (7.7 %) (Table 1).

PCR-mediated amplification of the 16S rRNA gene was carried out with primers 27f and 1492r (Sakamoto *et al.*, 2003). The 16S rRNA gene was sequenced directly from PCR products (van Berkum *et al.*, 1996) and a partial 16S rRNA gene sequence (1376 nt) for strain CCBAU 05354^T was obtained. The sequence obtained was deposited in the GenBank database and was compared with related sequences found in the database. The sequences were aligned using programs in the CLUSTAL_X package (Thompson *et al.*, 1997). A neighbour-joining tree was reconstructed and bootstrapped with 1000 replications of each sequence using MEGA version 3.1 (Kumar *et al.*, 2004). Isolate CCBAU 05354^T showed greatest sequence similarity (95.1 %) to *N. soli* JS13-8^T and showed lower levels of sequence similarity (<93.8 %) to the other species included in this analysis (Fig. 1).

Strain CCBAU 05354^T could be clearly differentiated from *N. soli* JS13-8^T on the basis of the novel strain's inability to hydrolyse Tween 80, and its ability to hydrolyse starch, ferment glucose and assimilate glycogen, L-fucose and DL-lactic acid. Additionally, CCBAU 05354^T could grow at pH 6.0–10.0 and displayed antibiotic resistance, while *N. soli* JS13-8^T grew at pH 5.0–8.0 and was sensitive to antibiotics. Their G+C contents were also different. Furthermore, the two strains differed in the percentages of the major cellular fatty acids present, and three fatty acids, iso-$C_{14:0}$ (1.0 %), $C_{14:0}$ (1.1 %) and $C_{16:1}\omega5c$ (1.1 %), were present in strain CCBAU 05354^T only.

On the basis of these results, strain CCBAU 05354^T represents a novel species of the genus *Niabella*, for which the name *Niabella yanshanensis* sp. nov. is proposed.

Description of *Niabella yanshanensis* sp. nov.

Niabella yanshanensis (yan.sha.nen′sis. N.L. fem. adj. *yanshanensis* of Yanshan, an emblem for Hebei Province, where the type strain was isolated).

Cells are aerobic, short rods, 0.4–0.6 μm in diameter and 0.7–1.7 μm long, non-motile and Gram-stain-negative. Colonies are orange in colour, convex and round with clear margins on NA agar. Growth occurs at 28 °C, at pH 6–10 and with 0–1 % NaCl (w/v). Resistant to ampicillin (5 μg ml^{-1}), gentamicin (5 μg ml^{-1}), kanamycin (5 μg ml^{-1}), chloramphenicol (5 μg ml^{-1}), neomycin (50 μg ml^{-1}) and erythromycin (100 μg ml^{-1}). Produces flexirubin pigment. Positive for catalase, oxidase, urease and glucose fermentation, and negative for nitrate reduction. Hydrolyses starch. Does not hydrolyse CM-cellulose, DNA, Tween 80, gelatin or phosphatidylcholine. Assimilates (in Biolog GN2 MicroPlate) α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, α-lactose, lactulose, maltose, D-mannose, melibiose, methyl β-D-glucoside, raffinose, L-rhamnose, sucrose, trehalose, turanose, acetic acid, D-galacturonic acid, D-glucuronic acid, DL-lactic acid and α-D-glucose 1-phosphate. Does not assimilate Tween 40, Tween 80, adonitol, D-arabitol, i-erythritol, *myo*-inositol, D-mannitol, D-psicose, D-sorbitol, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine,

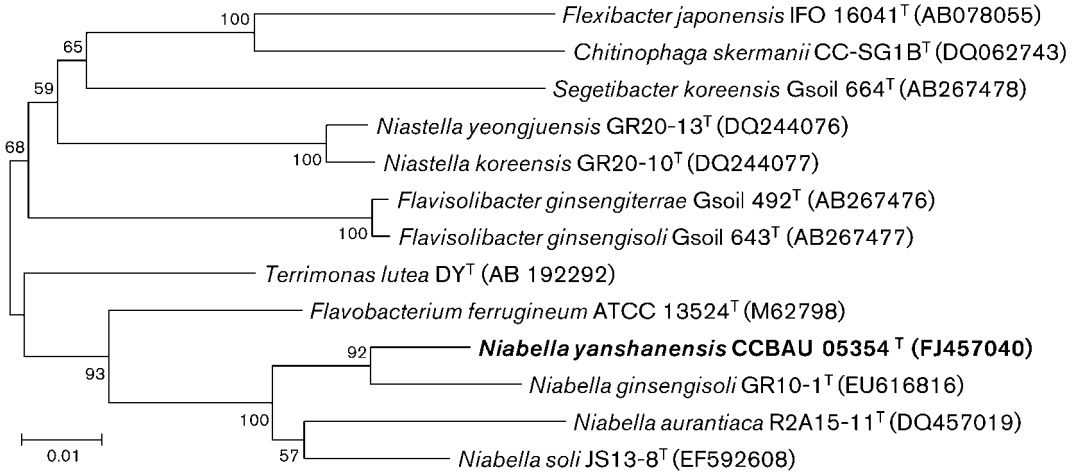

Fig. 1. Neighbour-joining phylogenetic tree for strain CCBAU 05354^T (FJ457040) and related strains, based on 16S rRNA gene sequences. Bootstrap values are shown as percentages of 1000 replicates. Bar, 1 substitution per 100 nucleotide positions.

L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenyethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol or DL-α-glycerol phosphate. Whether it can assimilate D-glucose 6-phosphate is indeterminate. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 7.8 %). The DNA G+C content is 42 mol%.

The type strain, CCBAU 05354^T (=LMG 24661^T =HAMBI 3031^T), was isolated from the soybean rhizosphere in Hebei Province, People's Republic of China.

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