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

Hao Wang,^{1,2} Yun Zeng Zhang,¹ Chao Xin Man,³ Wen Feng Chen,¹ Xin Hua Sui,¹ Ying Li,¹ Xiao Xia Zhang⁴ and Wen Xin Chen¹

Correspondence

Wen Xin Chen
wenxin@cau.edu.cn

¹State Key Laboratory of Agrobiotechnology and Department of Microbiology and Immunology, College of Biological Sciences, China Agricultural University, Beijing 100193, PR China

²The National Research Center of Soybean Engineering and Technology, Northeast Agricultural University, Harbin, PR China

³Heilongjiang Dairy Industry Technical Development Center, Northeast Agricultural University, Harbin, PR China

⁴Agricultural Cultural Collection of China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China

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}ω7c and/or C_{16:1}ω6c). 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}ω7c).

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/DBJ 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} ω5c	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}ω7c and/or C_{16:0}ω6c; b, iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

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}ω7c and/or C_{16:1}ω6c, 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}ω5c (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, phenylethylamine, 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} ω 7c and/or C_{16:1} ω 6c, 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.

References

- De Ley, J. (1970). Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J Bacteriol* **101**, 738–754.
- Kim, B. Y., Weon, H.-Y., Yoo, S.-H., Hong, S.-B., Kwon, S.-W., Stackebrandt, E. & Go, S.-J. (2007). *Niabella aurantiaca* gen. nov., sp. nov., isolated from a greenhouse soil in Korea. *Int J Syst Evol Microbiol* **57**, 538–541.

Kumar, S., Tomura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Reichenbach, H. (1992). The order *Cytophagales*. In *The Prokaryotes*, 2nd edn, vol. 4, pp. 3631–3675. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Sakamoto, M., Hayashi, H. & Benno, Y. (2003). Terminal restriction fragment length polymorphism analysis for human fecal microbiota and its application for analysis of complex bifidobacterial communities. *Microbiol Immunol* **47**, 133–142.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

van Berkum, P., Beyene, B. & Eardly, B. D. (1996). Phylogenetic relationships among *Rhizobium* species nodulating the common bean (*Phaseolus vulgaris* L.). *Int J Syst Bacteriol* **46**, 240–244.

Weon, H. Y., Kim, B.-Y., Joa, J.-H., Kwon, S.-W., Kim, W.-G. & Koo, B.-S. (2008). *Niabella soli* sp. nov., isolated from soil from Jeju Island, Korea. *Int J Syst Evol Microbiol* **58**, 467–469.