Niabella soli sp. nov., isolated from soil from Jeju Island, Korea

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A dark yellow-coloured bacterium, JS13-8^T, was isolated from a soil sample from Jeju Island, Republic of Korea. The cells were aerobic, Gram-negative, non-motile, short rods (0.5–0.7×0.8–1.4 µm). Growth occurred at 15–35 °C (optimally at 30 °C), at pH 5.0–8.0 (optimally at pH 6.0–7.0) and at 0–1 % NaCl (w/v). Flexirubin pigment was produced. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JS13-8^T was closely related to *Niabella aurantiaca* KACC 11698^T (95.0 % sequence similarity). The major respiratory quinone system was MK-7 and 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. The DNA G+C content was 45 mol%. On the basis of the phylogenetic, physiological and chemotaxonomic data, strain JS13-8^T represents a novel species of the genus *Niabella*, for which the name *Niabella soli* sp. nov. is proposed. The type strain is strain JS13-8^T (=KACC 12604^T=DSM 19437^T).

The genus *Niabella* was proposed by Kim *et al.* (2007) for a bacterium isolated from soil. This genus was characterized as Gram-negative, aerobic, non-flagellated, flexirubin-pigment-producing bacteria comprising short rods. Chemotaxonomically, the genus contains MK-7 as the predominant isoprenoid quinone and iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and summed feature 3 as the major fatty acids. The type species of the genus is *Niabella aurantiaca*.

In the course of a study on the bacterial population of a soil sample from Jeju, Republic of Korea, we isolated a dark yellow bacterial strain. The soil sample was serially diluted with 0.85 % NaCl (w/v) and the dilutions plated onto R2A agar (Difco). The strain was isolated after incubation for 4 days at 28 °C.

Phenotypic characteristics, including Gram-staining, catalase activity, oxidase activity and hydrolysis of carboxymethylcellulose, casein, chitin, DNA, hypoxanthine, tyrosine, Tween 80, starch and xanthine, were performed 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 under anaerobic conditions was tested in a GasPak (BBL) jar at 28 °C for 15 days on R2A medium. Growth at different temperatures and pH values was tested at between 5 and 45 °C (using increments of 5 °C) and pH 4–10 (using increments of 1 pH unit). Salt tolerance was tested on R2A broth supplemented with 0, 1, 2, 3 and 5 % (w/v) NaCl. Flexirubin pigment was detected from a colour shift after exposure to a 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). Strain JS13-8^T can grow on R2A, nutrient agar (Difco) and trypticase soy agar (Difco), but not on MacConkey agar (Difco). Phenotypic comparisons between strain JS13-8^T and *N. aurantiaca* KACC 11698^T are shown in Table 1.

The presence of isoprenoid quinones was investigated using HPLC, as described previously (Groth *et al.*, 1996). The cellular fatty acids of strain JS13-8^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 the Sherlock Microbial Identification System (MIDI, 1999). The G+C content of the chromosomal DNA was analysed as described by Mesbah *et al.* (1989) and was found to be 45 mol%. The major respiratory quinone system of strain JS13-8^T was MK-7.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JS13-8^T is EF592608.

Table 1. Phenotypic comparisons between strain JS13-8^T and *N. aurantiaca* KACC 11698^T

Data for *N. aurantiaca* KACC 11698^T were taken from Kim *et al.* (2007). +, Positive; –, negative. Fatty acids that account for <0.5 % of the total are not shown.

Characteristic	JS13-8 ^T	<i>N. aurantiaca</i> KACC 11698 ^T
Catalase/oxidase	+ / +	+ / –
Indole production	–	+
Casein hydrolysis	–	+
Assimilation of:		
D-Mannitol	+	–
Potassium gluconate	+	–
D-Ribose	+	–
Fatty acids (% of total)*		
anteiso-C _{15:0}	1.2	1.6
iso-C _{15:0}	29.2	33.7
iso-C _{15:0} 3-OH	2.2	2.9
iso-C _{15:1} G	18.4	22.3
C _{16:0}	6.8	3.5
C _{16:0} 2-OH	1.3	
C _{16:0} 3-OH	2.2	2.4
iso-C _{17:0} 3-OH	11.8	15.5
C _{18:0}	3.8	
C _{18:1} ω7c	1.5	
C _{18:1} ω9c	1.0	
Summed feature 3*	11.1	10.6
Summed feature 5*	3.4	
Unknown: ECL 13.565	1.1	
Unknown: ECL 16.582		1.2

*Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; summed feature 5 comprises anteiso-C_{18:0} and/or C_{18:2}ω6,9c.

The major cellular fatty acids were iso-C_{15:0} (29.2 %), iso-C_{15:1} G (18.4 %), iso-C_{17:0} 3-OH (11.8 %) and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, 11.1 %) (Table 1).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products were

carried out as described by Kwon *et al.* (2003): a partial 16S rRNA gene sequence (1396 nt) for strain JS13-8^T was obtained. 16S rRNA gene sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994). A phylogenetic analysis was performed using MEGA, version 3.1 (Kumar *et al.*, 2004). The phylogenetic tree was inferred using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Bootstrap analysis based on 1000 replications was undertaken to test the robustness of the phylogenetic tree (Felsenstein, 1985). Isolate JS13-8^T showed the highest level of sequence similarity (95.0 %) with respect to *N. aurantiaca* KACC 11698^T but showed low levels of sequence similarity (<92 %) with respect to other species included in this analysis. The phylogenetic tree also showed that strain JS13-8^T was grouped with *N. aurantiaca* KACC 11698^T with 100 % bootstrap support (Fig. 1). All branches of neighbour-joining tree were also present in the maximum-parsimony tree (data not shown).

Strain JS13-8^T can be clearly differentiated from *N. aurantiaca* on the basis of the novel strain's positive reaction for oxidase, its inability to produce indole or hydrolyse casein, its ability to assimilate D-mannitol, potassium gluconate and D-ribose and by the presence of some fatty acids such as C_{18:0} (3.8 %) and summed feature 5 (3.4 %).

On the basis of these results, strain JS13-8^T represents a novel species of the genus *Niabella*, for which the name *Niabella soli* sp. nov. is proposed.

Description of *Niabella soli* sp. nov.

Niabella soli (so'li. L. gen. n. *soli* of soil).

Colonies are dark yellow in colour, convex and round with clear margins. Cells are non-motile, Gram-negative, short rods that are 0.5–0.7 µm in diameter and 0.8–1.4 µm in length. Growth occurs at 15–35 °C (optimally at 30 °C), at pH 5.0–8.0 (optimally at pH 6.0–7.0) and with 0–1 % NaCl (w/v). Produces flexirubin pigment. Hydrolyses tyrosine and Tween 80. Does not hydrolyse casein, chitin, carboxymethylcellulose, DNA, hypoxanthine, starch or xanthine. Assimilates (in API 20NE and API ID 32GN strips) D-glucose, L-arabinose, D-mannose, N-acetylglucosamine,

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain JS13-8^T. Bootstrap values are shown as percentages of 1000 replicates. Bar, 2 substitutions per 100 nt.

maltose, L-rhamnose, sucrose, salicin and melibiose. Does not assimilate 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 or L-proline. Positive (in API 20NE and API ZYM strips) for aesculin hydrolysis, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase, and negative for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, lipase (C14), valine arylamidase, cystine arylamidase, trypsin and α -chymotrypsin. The predominant isoprenoid quinone is MK-7. 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. The DNA G + C content is 45 mol%.

The type strain, JS13-8^T (=KACC 12604^T=DSM 19437^T), was isolated from soil from Jeju Island, Republic of Korea.

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