Nevskia soli sp. nov., isolated from soil cultivated with Korean ginseng

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A Gram-negative bacterium, designated strain GR15-1^T, was isolated from a field cultivated with Korean ginseng. Cells were strictly aerobic, motile with multipolar flagella and rod-shaped. The strain grew optimally at 25–28 °C and pH 6.0–7.0. The predominant fatty acids of strain GR15-1^T were C_{18:1}ω7c, C_{16:0} and summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} l). The major isoprenoid quinone was ubiquinone 8. The G + C content of the genomic DNA was 63.9 mol%. Phylogenetic analysis showed that strain GR15-1^T formed a phyletic cluster with *Nevskia ramosa* Soe1^T, with a 16S rRNA gene sequence similarity of 96.8%. Based on phenotypic, chemotaxonomic and phylogenetic features, strain GR15-1^T represents a novel species within the genus *Nevskia*, for which the name *Nevskia soli* sp. nov. is proposed. The type strain is GR15-1^T (=KACC 11703^T =DSM 19509^T).

Although the genus *Nevskia* were first reported by Famintzin (1892), pure cultures were not available for many years. Stürmeyer *et al.* (1998) isolated pure cultures from a bog lake and a freshwater ditch, and some taxonomic properties were elucidated. The genus *Nevskia* was classified into the family *Xanthomonadaceae* within the *Gammaproteobacteria*. This genus, with the genus *Hydrocarboniphaga*, formed a deeply branched cluster separable from other member genera of the family *Xanthomonadaceae* such as *Xanthomonas*, *Rhodanobacter* and *Stenotrophomonas* (Stürmeyer *et al.*, 1998; Palleroni *et al.*, 2004).

In the course of the study of bacterial diversity in fields cultivated with Korean ginseng (*Panax ginseng* C. A. Meyer), a novel bacterial strain, GR15-1^T, was isolated in the Yeongju region of Korea. Phylogenetically, this strain clustered with *Nevskia ramosa* and *Hydrocarboniphaga effusa*.

To investigate the morphological and some physiological characteristics of strain GR15-1^T, the strain was routinely cultivated on R2A medium at 28 °C. Unless otherwise indicated, phenotypic characteristics were studied using standard procedures (Smibert & Krieg, 1994; Weon *et al.*, 2006). After 3 days incubation at 28 °C, cell morphology was examined by light microscopy (AXIO; Zeiss) and transmission electron microscopy (TEM). For TEM

observation, cells were negatively stained with 0.5 % (w/v) uranyl acetate and examined with an LEO model 912AB electron microscope. For the various physiological tests, API 20NE and API ID 32GN test strips (bioMérieux) were used according to the manufacturer's instructions.

Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified product were carried out as described previously (Weon *et al.*, 2006). The resultant 16S rRNA gene sequence (1421 bp) was aligned with corresponding sequences of some members classified in the family *Xanthomonadaceae* retrieved from the GenBank database using the CLUSTAL W program (Thompson *et al.*, 1994). A phylogenetic tree was produced using the software package MEGA version 3.1 (Kumar *et al.*, 2004). Distances (using distance options according to Kimura's two-parameter model) and clustering using the neighbour-joining and maximum-parsimony methods were determined by using bootstrap values based on 1000 replicates.

The G + C content of the genomic DNA was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18S; Supelco). Cellular fatty acids were determined for a culture grown on R2A at 28 °C for 2 days and were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser, 1990). Isoprenoid quinones were examined according to Groth *et al.* (1996).

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain GR15-1^T is EF178286.

Fig. 1. Morphology of cells of strain GR15-1^T after growth for 3 days on R2A. Transmission electron micrograph (a) and phase-contrast micrograph (b). Bars, 500 nm (a) and 10 µm (b).

Strain GR15-1^T grew on R2A, but did not grow on nutrient agar, trypticase soy agar or MacConkey agar (all from Difco). Cells of GR15-1^T were motile with multiple polar flagella and rod-shaped, 0.5 µm wide and 1.5–2.0 µm long

Table 1. Phenotypic characteristics that differentiate strain GR15-1^T from other related type strains

Strains: 1, GR15-1^T; 2, *N. ramosa* DSM 11499^T; 3, *H. effusa* DSM 16095^T. Data are from this study unless indicated. All strains are strictly aerobic, Gram-negative and motile and do not assimilate trisodium citrate. All strains hydrolyse Tween 80. All strains are negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and aesculin hydrolysis (API 20 NE test strips). None of the strains assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, phenylacetic acid, L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, melibiose, L-fucose, D-sorbitol, valeric acid, L-histidine, potassium 2-ketogluconate, 4-hydroxybenzoic acid or L-proline (API 20 NE and API ID 32GN test strips). +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3
Flagella	Multipolar	ND	One polar, subpolar or, more rarely, lateral insertion ^{a*}
Catalase/oxidase	+/+	w/– ^b	ND/ND
Gelatin hydrolysis	–	+	+
β-Galactosidase	–	+	–
Assimilation of:			
Malic acid	–	–	+
Sucrose	–	–	+
Lactic acid	–	–	+
Glycogen	+	–	+
Propionic acid	–	–	+
3-Hydroxybutyric acid	–	–	+
DNA G + C content (mol%)	63.9	67.8 ^b	60–61 ^a

*Data from: a, Palleroni *et al.* (2004); b, Stürmeyer *et al.* (1998).

(Fig. 1). Phenotypic characteristics that differentiate strain GR15-1^T from related species are listed in Table 1.

According to the neighbour-joining tree (Fig. 2), strain GR15-1^T was placed within a cluster of two uncultured bacteria, uncultured eubacterium WD284 and uncultured rape rhizosphere bacterium wr0008, which were respectively cloned from polychlorinated biphenyl-polluted soil (Nogales *et al.*, 2001) and the rhizoplane of oilseed rape (Kaiser *et al.*, 2001). This cluster forms a deeply branching clade with another cluster including *N. ramosa* Soel^T on the basis of a considerable bootstrap value (86%). Strain GR15-1^T shares 96.8% 16S rRNA gene sequence identity with *N. ramosa* Soel^T and 94.2% sequence identity with *H. effusa* AP103^T. The maximum-parsimony tree showed a

Table 2. Cellular fatty acid compositions of strain GR15-1^T and related strains

Strains: 1, GR15-1^T; 2, *N. ramosa* DSM 11499^T; 3, *H. effusa* DSM 16095^T. Data are from this study. Cells for all strains were harvested after growth on R2A at 28 °C for 2 days. Fatty acids representing less than 1.0% were omitted.

Fatty acid	1	2	3
C _{12:0}	7.6	6.1	9.1
C _{12:0} 3-OH	–	2.7	1.4
C _{14:0}	5.3	11.8	2.3
C _{14:0} 2-OH	1.0	4.5	1.9
C _{16:0}	17.5	8.9	18.1
C _{16:0} 3-OH	2.2	–	–
C _{16:0} N alcohol	1.1	–	–
iso-C _{16:0}	1.2	–	–
C _{17:0} cyclo	6.3	–	–
C _{18:1} 2-OH	–	3.3	–
C _{18:1} ω7c	31.1	28.7	44.7
C _{19:0} ω8c cyclo	3.9	–	–
Summed feature 2*	10.3	2.0	1.7
Summed feature 3*	9.9	29.2	16.4

*Summed feature 2 comprises C_{14:0} 3-OH and/or iso-C_{16:1} I. Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1} ω7c.

Fig. 2. Neighbour-joining phylogenetic tree (Saitou & Nei, 1987) based on 16S rRNA gene sequences, showing the position of strain GR15-1^T. Bootstrap values are shown as percentages of 1000 replicates (only values above 50% are shown). GenBank accession numbers are shown in parentheses. Bar, 0.02 substitutions per nucleotide position.

very similar topology for the novel strain and other related species (not shown).

The DNA G + C content of strain GR15-1^T was 63.9 mol%. Major cellular fatty acids (>10%) identified from strain GR15-1^T were C_{18:1}ω7c, C_{16:0} and summed feature 2. The full fatty acid profile is detailed in Table 2. The major isoprenoid quinone was ubiquinone 8 (Q-8).

Strain GR15-1^T can be clearly differentiated from its closest relative, *N. ramosa* DSM 11499^T, by means of some phenotypic properties such as the production of oxidase and the inability to hydrolyse gelatin or to produce β-galactosidase and by differences in the fatty acid compositions (Tables 1 and 2). However, molecular data such as its high 16S rRNA gene sequence similarity and similar G + C content confirmed the affiliation of strain GR15-1^T to the genus *Nevskia*. On the basis of the phylogenetic evidence together with the phenotypic characteristics presented in this study, strain GR15-1^T represents a novel species within the genus *Nevskia*, for which the name *Nevskia soli* sp. nov. is proposed.

Description of *Nevskia soli* sp. nov.

Nevskia soli (so'li. L. neut. gen. n. *soli* of soil, the source of the type strain).

Cells are Gram-negative, motile, non-spore-forming and rod-shaped (0.5 × 1.5–2.0 μm). Colonies are white, circular and convex with clear margins after 4 days on R2A medium. Growth occurs at 15–30 °C (optimum 25–28 °C) and pH 4.0–7.0 (optimum pH 6.0–7.0). Grows in the absence of NaCl, but does not grow on 2% NaCl. Tween 80 and starch are hydrolysed. Hydrolysis of casein, chitin, CM-cellulose, DNA, pectin and tyrosine is not observed. Major isoprenoid quinone is Q-8. Predominant cellular fatty acids (>10%) are C_{18:1}ω7c, C_{16:0} and summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} I). DNA G + C content of the type strain is 63.9 mol% (HPLC).

The type strain, GR15-1^T (=KACC 11703^T =DSM 19509^T), was isolated from soil cultivated with Korean ginseng in the Yeongju region of Korea.

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