Nevskia terrae sp. nov., isolated from soil

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A Gram-negative bacterium, designated KIS13-15^T, was isolated from soil in Korea. Cells were strictly aerobic rods and were motile with 1–3 subpolar flagella. 16S rRNA gene sequence analysis revealed that strain KIS13-15^T belonged to the order *Xanthomonadales* of the class *Gammaproteobacteria* and was closely related to *Nevskia soli* GR15-1^T (97.9% 16S rRNA gene sequence similarity) and *Nevskia ramosa* Soe1^T (96.8%). Strain KIS13-15^T exhibited 38 and 29% DNA–DNA relatedness with *N. soli* KACC 11703^T and *N. ramosa* DSM 11499^T, respectively. The major fatty acids (>10% of the total) were C_{18:1}ω7c (40.6%) and C_{16:0} (12.4%). The major isoprenoid quinone was ubiquinone 8. DNA G+C content was 67.3 mol%. The phenotypic characterization combined with 16S rRNA gene sequence analysis and DNA–DNA hybridization clearly classified strain KIS13-15^T in a novel species of the genus *Nevskia*, for which the name *Nevskia terrae* sp. nov. is proposed. The type strain is KIS13-15^T (=KACC 12736^T =JCM 15425^T).

The genus *Nevskia* is classified in the order *Xanthomonadales* within the class *Gammaproteobacteria*. At the time of writing, the genus *Nevskia* included two species, *Nevskia ramosa* and *Nevskia soli*. *N. ramosa* was first isolated from the water surface of an aquarium in a botanical garden (Famintzin, 1892). As pure cultures were not available, little was known about the properties of the genus until Stürmeyer *et al.* (1998) isolated pure cultures of *N. ramosa* from a bog lake and a freshwater ditch and provided ecophysiological and phylogenetic descriptions. *N. soli* was isolated from a field cultivated with Korean ginseng (Weon *et al.*, 2008). Chemotaxonomically, ubiquinone 8 (Q-8) is the predominant isoprenoid quinone in this genus and C_{18:1}ω7c is the major fatty acid. This paper describes a new bacterial isolate, KIS13-15^T, which belongs to the class *Gammaproteobacteria* and has a 16S rRNA sequence similar to those of the genus *Nevskia*.

Strain KIS13-15^T was isolated as part of a study of bacterial diversity in 30 islands of Korea. Soil samples were serially diluted with 0.85% (w/v) NaCl and appropriate 10-fold dilutions were plated on R2A agar (Difco; Reasoner & Geldreich, 1985). After incubation at 28 °C for 5 days, strain KIS13-15^T was isolated from a sample collected from Baekryeung Island (37° 58' 47" N 124° 42' 50" E) in the West Sea, Republic of Korea. The soil sample had the following chemical properties: pH 4.4, 40.3 g total organic

carbon kg⁻¹, electrical conductivity 0.65 dS m⁻¹, 527.9 mg available P₂O₅ kg⁻¹, 0.21 cmol exchangeable K kg⁻¹, 2.5 cmol Ca kg⁻¹ and 0.6 cmol Mg kg⁻¹. The reference strains *N. soli* KACC 11703^T and *N. ramosa* DSM 11499^T were incubated on R2A agar according to conditions described by Weon *et al.* (2008). Colonies of strain KIS13-15^T were distinguishable on R2A agar because of their ivory colour.

After cultivation on R2A agar at 28 °C for 8 days, cell morphology and motility of strain KIS13-15^T were observed using phase-contrast microscopy (Axio; Zeiss) and transmission electron microscopy (912AB; LEO). Gram staining, presence of catalase and oxidase, accumulation of poly-β-hydroxybutyrate and hydrolysis of casein, Tween 80, DNA and starch were determined as described by Smibert & Krieg (1994). Hydrolysis of CM-cellulose (0.1%, w/v), chitin from crab shells (1%, w/v), pectin (0.5%, w/v) and tyrosine (0.5%, w/v) was also examined. Growth was determined under the following conditions in R2A broth at 28 °C after 20 days unless otherwise stated: at 5–45 °C (in increments of 5 °C) and 28 °C; at pH 4–10 (in increments of one pH unit), using citrate/phosphate or Tris/HCl buffers (Breznak & Costilow, 1994) to adjust the pH; and with 0–5% (w/v) NaCl (in increments of 1% NaCl) and 7% NaCl. Carbon source utilization tests were performed with microtitre plates as described by Kämpfer *et al.* (1991). Additional enzyme activities and biochemical features were tested using the API 20 E, API 20 NE and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Cells of strain KIS13-15^T were strictly aerobic, Gram-negative rods

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KIS13-15^T is GQ845011.

A supplementary figure is available with the online version of this paper.

(0.4–0.6 µm wide and 1.0–2.5 µm long) and motile with 1–3 subpolar flagella (Supplementary Fig. S1, available in IJSEM Online). Strain KIS13-15^T was able to grow on R2A agar, but not on nutrient agar (NA), tryptic soy agar (TSA) or MacConkey agar (Difco). The phenotypic features of strain KIS13-15^T are presented in Table 1.

Genomic DNA was isolated by the method of Ausubel *et al.* (1987), except that the lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA gene was amplified using the universal primers fD1 and rP2 (Weisburg *et al.*, 1991) and sequenced as described by Kwon *et al.* (2003). Phylogenetic neighbours were identified and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The 16S rRNA gene sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) and phylogenetic analysis was performed using MEGA version 3.1 (Kumar *et al.*, 2004). Distances were calculated using distance options from the Kimura two-parameter model (Kimura, 1983) and clustering was performed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values were based on 1000 replicates (Felsenstein, 1985). A nearly complete 16S rRNA gene sequence (1461 bp) of strain KIS13-15^T was determined and revealed that strain KIS13-15^T was most closely related to *N. soli* GR15-1^T (97.9% 16S rRNA gene sequence similarity) and *N. ramosa* Soe1^T (96.8%). Lower sequence similarities were found with sequences from members of other genera, for example *Hydrocarboniphaga effusa* AP103^T (95.0%) and *Alkanibacter difficilis* MN154.3^T (93.7%). The neighbour-joining tree showed that strain KIS13-15^T formed a compact cluster with *N. soli* GR15-1^T and *N. ramosa* Soe1^T that had 99% bootstrap support, which was also recovered in the tree generated with the maximum-parsimony method (Fig. 1).

After cells of strain KIS13-15^T had been prepared on R2A agar for 2 days at 28 °C, fatty acid methyl esters were extracted and prepared using the standard protocol of the Microbial Identification System (MIDI). Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). The DNA G+C content of strain KIS13-15^T was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18 S; Supelco). DNA–DNA hybridization was carried out using the filter hybridization method as described by Seldin & Dubnau (1985). Probes were labelled using the non-radioactive DIG High Prime system and hybridized DNA was visualized using the DIG Luminescent Detection kit (Roche). DNA–DNA relatedness was quantified using a densitometer (Bio-Rad). The predominant fatty acids of strain KIS13-15^T were C_{16:0} (12.4%) and C_{18:1ω7c} (40.6%) (Table 2). The predominant isoprenoid quinone of strain KIS13-15^T was Q-8 (93.4%) and the minor isoprenoid quinones were Q-7 (2.1%), Q-9 (13.0%) and Q-10 (3.3%). The DNA G+C content was 67.3 mol%. Strain KIS13-15^T exhibited 38 and 29% DNA–DNA relatedness with *N. soli* KACC 11703^T

Table 1. Differential characteristics among strain KIS13-15^T and its closest phylogenetic neighbours

Strains: 1, KIS13-15^T; 2, *N. soli* KACC 11703^T; 3, *N. ramosa* DSM 11499^T. Data were taken from this study unless otherwise indicated. All strains are strictly aerobic, Gram-negative, motile and positive for Tween 80 hydrolysis, assimilation of *N*-acetylglucosamine, inositol, mannitol, lactate, proline and propionate and production of esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. All strains are negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and aesculin hydrolysis, assimilation of glucose, sucrose, salicin, 5-ketogluconate, 2-ketogluconate, adipate, itaconate, malonate and phenylacetate and production of lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive; (+), weakly positive; –, negative; ND, no data available

Characteristic	1	2	3
Flagella	>1, subpolar, or, more rarely, polar and lateral insertion	Multipolar*	ND*
Catalase	+	+	(+)
Oxidase	–	+	–†
Starch hydrolysis	–	+	(+)
Gelatin hydrolysis	–	–	+
Assimilation of:			
L-Arabinose	+	–	–
Ribose	–	+	–
Mannose	–	–	+
Rhamnose	+	+	–
Sorbitol	+	+	–
Maltose	–	–	+
Melibiose	+	–	–
Glycogen	–	+	–
L-Fucose	+	+	–
Gluconate	+	+	–
3-Hydroxybutyric acid	+	+	–
Acetate	+	–	+
Citrate	+	–	–
Histidine	+	–	–
Malate	+	–	+
Enzymes (API ZYM)			
Alkaline phosphatase	–	+	+
β-Galactosidase	–	–	+
DNA G+C content (mol%)	67.3	63.9*	67.8†

*Data from Weon *et al.* (2008).

†Data from Stürmeyer *et al.* (1998).

and *N. ramosa* DSM 11499^T, respectively, which was below the threshold value (70%) suggested for species delineation (Wayne *et al.* 1987) and indicated that strain KIS13-15^T represented a novel species of the genus *Nevskia*.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain KIS13-15^T and strains of closely related species. Filled circles indicate that the corresponding nodes were recovered in the maximum-parsimony tree. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. Bar, 0.02 substitutions per nucleotide position.

Strain KIS13-15^T can be differentiated from *N. soli* KACC 11703^T on the basis of the absence of oxidase production, starch hydrolysis, alkaline phosphatase activity and ribose and glycogen assimilation. Strain KIS13-15^T can be differentiated from *N. ramosa* DSM 11499^T on the basis of the absence of starch and gelatin hydrolysis, β -galactosidase and alkaline phosphatase activity and mannose and maltose assimilation. The fatty acid composition

of KIS13-15^T was similar to that of *N. soli* GR15-1^T; however, strain KIS13-15^T could be differentiated from *N. ramosa* DSM 11499^T. The major fatty acids (>10% of the total) of strain KIS13-15^T were C_{18:1} ω 7c and C_{16:0}, whereas those of *N. ramosa* DSM 11499^T were summed feature 3, C_{18:1} ω 7c and C_{14:0}.

On the basis of phenotypic, chemotaxonomic and phylogenetic analysis, strain KIS13-15^T represents a novel species within the genus *Nevskia*, for which the name *Nevskia terrae* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions of KIS13-15^T and other species of the genus *Nevskia*

Strains: 1, KIS13-15^T; 2, *N. soli* GR15-1^T (data from Weon *et al.*, 2008); 3, *N. ramosa* DSM 11499^T (Weon *et al.*, 2008). Cells of strain KIS13-15^T were harvested after cultivation on R2A agar at 28 °C for 2 days. Values are percentages of total fatty acids. —, Less than 1% or not detected.

Fatty acid (%)	1	2	3
C _{12:0}	8.1	7.6	6.1
C _{12:0} 3-OH	—	—	2.7
C _{14:0}	8.5	5.3	11.8
C _{14:0} 2-OH	—	1.0	4.5
C _{16:0}	12.4	17.5	8.9
C _{16:0} 3-OH	1.7	2.2	—
C _{16:0} N alcohol	—	1.1	—
iso-C _{16:0}	1.3	1.2	—
C _{17:0} cyclo	—	6.3	—
C _{18:1} 2-OH	2.8	—	3.3
C _{18:1} ω 7c	40.6	31.1	28.7
C _{19:0} ω 8c cyclo	3.2	3.9	—
Summed features*			
2	8.7	10.3	2.0
3	8.7	9.9	29.2

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 consisted of C_{14:0} 3-OH and/or iso-C_{16:1} I. Summed feature 3 consisted of iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c.

Description of *Nevskia terrae* sp. nov.

Nevskia terrae (ter'rae. L. gen. n. *terrae* of the soil).

Cells are strictly aerobic, Gram-negative rods (0.4–0.6 μ m in width and 1.0–2.5 μ m in length). Motile with 1–3 subpolar flagella. Catalase-positive and oxidase-negative. Grows on R2A agar, but not NA, TSA or MacConkey agar. Grows very slowly and takes >1 week for colonies to fully develop. Colonies are ivory, circular and convex with clear margins after 8 days. Grows at 15–35 °C (optimum 28–30 °C) and at pH 5–7 (optimum pH 6–7). Does not grow with >1% NaCl. Hydrolyses Tween 80, but not casein, chitin, CM-cellulose, DNA, hypoxanthine, pectin, starch, tyrosine or xanthine. Utilizes L-arabinose, rhamnose, sorbitol, N-acetylglucosamine, melibiose, L-fucose, gluconate, inositol, D-mannitol, 3-hydroxybutyric acid, acetate, citrate, histidine, lactate, malate, proline and propionate as sole carbon and energy sources, but does not utilize D-ribose, D-glucose, D-mannose, maltose, sucrose, glycogen, 5-ketogluconate, salicin, 2-ketogluconate, adipate, itaconate, malonate or phenylacetate. With API ZYM, positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for alkaline phosphatase, lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Voges–Proskauer test is

negative. With API 20 E, negative for H₂S production, citrate utilization and acid production from D-glucose, D-mannitol, D-sorbitol, L-rhamnose, inositol, sucrose, melibiose, amygdalin and L-arabinose. With API 20 NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and aesculin hydrolysis. The major fatty acids (>10%) are C_{18:1ω7c} and C_{16:0}. Ubiquinone-8 is the predominant quinone. The DNA G+C content of the type strain is 67.3 mol%.

The type strain, KIS13-15^T (=KACC 12736^T =JCM 15425^T), was isolated from soil of Baekryoung Island, Republic of Korea.

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