Isoptericola nanjingensis sp. nov., a mineral-weathering bacterium

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A Gram-positive-staining, non-motile, rod- or coccoid-shaped actinobacterium, designated strain H17^T, was isolated from a soil sample from Nanjing, Jiangsu Province, PR China. The organism grew optimally at 30 °C, pH 7.0 and with 3% NaCl (w/v). Strain H17^T contained L-Lys–D-Asp as the cell-wall peptidoglycan type and galactose, xylose and mannose as the whole-cell sugars. The major fatty acids were anteiso-C_{15:0} and iso-C_{15:0}. The total polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phospholipids, phosphatidylinositol, phosphoglycolipid, one unidentified glycolipid and one unidentified lipid. The menaquinone was MK9(H₄). Mycolic acids were not detected. The DNA G+C content was 72.4 mol%. Phylogenetic analysis of this strain based on 16S rRNA gene sequences revealed 97.8–99.6% similarity to recognized species of the genus *Isoptericola*. The low level of DNA–DNA relatedness to other species of the genus *Isoptericola* and the many phenotypic properties that distinguished strain H17^T from recognized species of this genus demonstrated that isolate H17^T should be classified as representing a novel species of the genus *Isoptericola*. The name *Isoptericola nanjingensis* sp. nov. is proposed for this novel species. The type strain is H17^T (=DSM 24300^T=CCTCC AB 2011005^T).

The genus *Isoptericola* was proposed by Stackebrandt *et al.* (2004) to accommodate an atypical species of the genus *Cellulosimicrobium*, *Cellulosimicrobium variabile* (Bakalidou *et al.*, 2002). Currently, the genus *Isoptericola* comprises six species with validly published names: *Isoptericola variabilis* (Stackebrandt *et al.*, 2004), *I. hypogaeus* (Groth *et al.*, 2005), *I. halotolerans* (Zhang *et al.*, 2005), *I. dokdonensis* (Yoon *et al.*, 2006), *I. jiangsuensis* (Wu *et al.*, 2010) and *I. chiayiensis* (Tseng *et al.*, 2011). In this study, a bacterial strain, H17^T, was characterized that was phylogenetically closely related to the genus *Isoptericola*.

Strain H17^T was isolated from a soil sample using the dilution plating method on a sucrose–mineral salts medium. This medium contained (l⁻¹): 10.0 g sucrose, 0.5 g yeast extract, 1 g (NH₄)₂SO₄, 2 g K₂HPO₄, 0.5 g MgSO₄, 0.1 g NaCl, 0.5 g CaCO₃ and 15 g agar. Strain H17^T was able to weather potassium-bearing minerals. Morphology and motility were examined by light microscopy (CX21; Olympus) and transmission electron microscopy (H-7650; Hitachi) using cells from the exponential phase. The determination of the optimal growth temperature was performed using trypticase soy agar (TSA) at 4, 10, 20, 37 and 42 °C. The pH range for

growth was determined in nutrient broth adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units) with HCl or Na₂CO₃. To investigate tolerance of NaCl, trypticase soy broth was used and the NaCl concentration was altered (0, 0.5, 1.0–10.0%, w/v, at intervals of 1.0%). Standard physiological tests were performed according to the methods described by Gordon *et al.* (1974) and Lányi (1987). Susceptibility to antibiotics was tested on TSA plates using discs containing the following concentrations of antibiotics: carbenicillin, 100 µg; chloramphenicol, 30 µg; gentamicin, 10 µg; kanamycin, 30 µg; lincomycin, 2 µg; midcamycin, 30 µg; neomycin, 30 µg; novobiocin, 30 µg; polymyxin B, 30 µg; rifampicin, 5 µg; spectinomycin, 100 µg; streptomycin, 10 µg; tetracycline, 30 µg and vancomycin, 30 U. Metabolic properties and enzyme activities were tested using API 50 CHB/E and API ZYM systems (bioMérieux) according to the manufacturer's instructions.

Growth of strain H17^T was aerobic and cells were Gram-stain positive. Both coccoid- and rod-shaped cells were observed, but neither flagella nor spores were found (see Fig. S1 in IJSEM Online). Colonies on TSA were pale yellow, with a smooth surface and diameter of 1.0–2.0 mm after incubation for 48 h at 30 °C. Strain H17^T could grow at 10–37 °C (optimum 30 °C), at pH 5.5–10 (optimum pH 7.0) and with 0.5–10% NaCl (w/v; optimum 3%). Detailed physiological and biochemical

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain H17^T is HQ222356.

Supplementary figures are available with the online version of this paper.

Table 1. Phenotypic characteristics of strain H17^T and other species of the genus *Isoptericola*

Strains: 1, H17^T; 2, *I. variabilis* MX5^T; 3, *I. hypogaeus* HKI 0342^T; 4, *I. jiangsuensis* CLG^T; 5, *I. dokdonensis* DS-3^T; 6, *I. halotolerans* YIM 70177^T; 7, *I. chiayiensis* 06182M-1^T. All taxa are positive for Gram-staining, catalase, hydrolysis of aesculin and starch, utilization of glycerol, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, arbutin, salicin, cellobiose, maltose, sucrose, starch, glycogen and for the activity of esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α - and β -glucosidase. All strains are susceptible to carbenicillin (100 μ g), chloramphenicol (30 μ g), gentamicin (10 μ g), neomycin (30 μ g), novobiocin (30 μ g), polymyxin B (30 μ g), roxithromycin (15 μ g), tetracycline (30 μ g) and vancomycin (30 U). All taxa are negative for motility, utilization of erythritol, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, D-sorbitol, xylitol, D-tagatose, D-fucose, L-arabitol, L-fucose, potassium gluconate, potassium 2- and 5-ketogluconate and are not susceptible to kanamycin (30 μ g). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6	7
Oxidase	+	+	–	–	+	+	–
Growth at/with:							
42 °C	–	+	–	–	–	–	–
10 % NaCl (w/v)	+	+	–	+	–	+	+
Hydrolysis of:							
Casein	–	+	+	–	–	–	–
Gelatin	–	+	+	–	+	–	–
Hypoxanthine	+	+	+	–	–	–	–
Tyrosine	–	+	–	–	–	–	–
Urea	–	+	–	–	–	–	–
Xanthine	+	+	+	–	–	–	–
Nitrate reduction	–	+	+	+	+	–	–
Methyl red	+	+	–	–	–	–	–
Indole	–	+	–	–	+	+	+
Utilization of:							
D-Arabinose	–	+	–	–	–	w	w
D-Ribose	+	+	–	+	–	+	w
L-Rhamnose	+	+	–	w	–	–	+
D-Mannitol	+	+	–	–	–	+	–
Lactose	+	+	–	+	–	–	–
Melibiose	+	–	–	+	–	–	–
Trehalose	+	–	–	+	+	–	w
Melezitose	+	+	–	+	–	–	–
Raffinose	+	+	–	+	–	–	–
Turanose	+	+	–	+	+	+	–
D-Lyxose	+	–	–	–	–	–	–
D-Arabitol	+	+	w	–	–	–	w
Methyl β -D-xylopyranoside	+	–	–	–	–	–	–
Methyl α -D-mannopyranoside	+	–	–	–	–	–	–
Methyl α -D-glucopyranoside	+	+	–	+	–	–	–
N-Acetylglucosamine	+	+	–	+	–	–	–
Amygdalin	+	+	+	+	–	+	–
Inulin	+	–	–	+	–	+	–
Gentiobiose	+	+	–	+	–	+	+
API ZYM tests:							
Alkaline phosphatase	+	+	–	+	+	–	+
Esterase (C4)	+	+	–	+	–	+	+
Lipase (C14)	+	+	–	+	w	w	+
Trypsin	–	+	–	+	+	+	+
α -Chymotrypsin	–	+	–	+	+	–	+
Acid phosphatase	–	–	–	+	+	–	–
α -Galactosidase	+	+	–	+	–	–	–
β -Glucuronidase	+	+	–	–	–	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
<i>N</i> -Acetyl- β -glucosaminidase	+	+	–	+	–	–	+
α -Mannosidase	+	+	+	–	–	–	–
α -Fucosidase	–	+	w	–	–	–	–
Susceptibility to:							
Lincomycin (2 μ g)	–	–	–	–	+	+	–
Midecamycin (30 μ g)	–	+	–	+	+	+	+
Rifampicin (5 μ g)	+	–	–	+	+	+	+
Spectinomycin (100 μ g)	–	–	+	+	–	+	–
Streptomycin (10 μ g)	+	–	+	+	+	+	+
Cell-wall sugars*	Gal, Xyl, Man	Gal, Glu, Rha	Gal, Glu, Pha, Man	Gal, Rha, Xyl	Gal, Glu, Rha, Rib	Gal	Glu, Gal, Rha
Peptidoglycan type	L-Lys-D-Asp	L-Lys-D-Asp	L-Lys-D-Glu	L-Lys-D-Asp	L-Lys-D-Asp	L-Lys-D-Asp	L-Lys-L-Ala-L-Glu-D-Asp
Polar lipids†	DPG, PG, PL, PI, one known PGL, one unknown glycolipid, one unknown lipid	DPG, PG, PL, PI	DPG, PG, PI, PIM, two unknown phospholipids, one unknown glycolipid	DPG, PG, PL, PI, three PGLs, one unidentified lipid	DPG, PG, PI, two unidentified glycolipids	DPG, PG, PI	DPG, PG, PI, two glycolipids, two unknown PGLs
DNA G + C (mol%)	72.4	70–72	73.8	70.3	74.1	72.8	72.8

*Gal, Galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.

†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, phosphoglycolipid; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, phospholipids.

characteristics of the strain are given in Table 1 and the species description.

The chemotaxonomic characteristics of strain H17^T were determined using cells cultured on TSA. The elucidation of the peptidoglycan structure was carried out as described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was used instead of paper chromatography. Quantitative analysis of amino acids was carried out by GC by using a EZ:faast kit (Phenomenex) according to the manufacturer's instructions. Calibration was achieved by using a standard composed of peptidoglycan relevant amino acids. For analysis of the cell-wall sugars, the cell-wall preparation was hydrolysed with 0.5 M H₂SO₄ (2 h at 100 °C). H₂SO₄ was removed by 20 % *N*, *N*-diocetyl-methylamine in chloroform according to Whiton *et al.* (1985). The sugars in the hydrolysate were analysed by TLC on cellulose plates according to Stanek & Roberts (1974). For polar lipid analysis, cell mass of strain H17^T was harvested from tryptic soy broth after incubation for 20 h at 30 °C. Polar lipids were extracted from 100 mg freeze-dried cell material using the two-stage method described by Tindall (1990a, b) and were identified using two-dimensional silica gel TLC followed by spraying with appropriate detection reagents (Art. No. 818 135; Macherey-Nagel). The polar lipid analysis was carried out by the DSMZ. For fatty acid methyl ester analysis, cell mass of strain H17^T was harvested from TSA plates after incubation for 2 days (exponential phase) at 30 °C. The fatty

acid methyl esters were extracted and prepared according to the protocol of Miller (1982) with minor modifications from Kuykendall *et al.* (1988). The fatty acid methyl ester mixtures were separated using the Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID), which consisted of a GC (6890N; Agilent) fitted with a 5 % phenyl-methyl silicone capillary column (0.2 mm $\times$ 25 m), a flame-ionization detector, an automatic sampler (7683A; Agilent) and a computer (Hewlett Packard) running the MIDI database. Menaquinones were extracted and purified by the methods of Collins *et al.* (1977) and Tamaoka *et al.* (1983) and then analysed by HPLC (1100; Agilent) with a Zorbax EclipseXDB-C18.5 column (250 $\times$ 4.6 mm). Mycolic acids were examined by TLC as described by Minnikin *et al.* (1980).

The total hydrolysate of the cell-wall peptidoglycan of strain H17^T contained alanine and glutamic acid with smaller amounts of lysine and aspartic acid. In addition, the peptide L-Lys-D-Asp was detected, which was stable under the hydrolysis conditions (100 °C, 4 M HCl, 16 h). Quantitative analysis of the peptidoglycan amino acids after hydrolysis under stronger conditions (120 °C, 6 M HCl, 16 h) by using the EZ:faast kit resulted in the following quantitative ratio: 0.6 Ala:0.3 Asp:1.0 Glu:0.5 Lys. The reduced amounts of lysine and aspartic acid were due to the occurrence of the stable peptide L-Lys-D-Asp. Partial hydrolysis (100 °C, 4 M HCl, 45 min) resulted in the additional peptides L-Ala-D-Glu, D-Asp-L-Lys-D-Ala and L-Lys-D-Ala. From these data,

Table 2. Fatty acid profiles (>1%) of strain H17^T and type strains of the genus *Isoptericola*

Strains: 1, H17^T; 2, *I. variabilis* MX5^T; 3, *I. hypogeus* HKI 0342^T; 4, *I. jiangsuensis* CLG^T; 5, *I. dokdonensis* DS-3^T; 6, *I. halotolerans* YIM 70177^T; 7, *I. chiayiensis* 06182M-1^T.

Fatty acid (%)	1	2	3	4	5	6	7
C _{14:0}	5.97	6.6	—	1.17	3.3	2.33	1.75
C _{15:0}	—	—	—	2.64	2.6	—	—
C _{16:0}	8.32	7.2	—	3.13	9.0	20.05	9.72
C _{17:0}	—	—	—	1.24	—	—	1.48
iso-C _{13:0}	—	—	—	3.98	1.1	—	—
iso-C _{14:0}	2.13	1.5	8.4	3.40	4.2	—	3.01
iso-C _{15:0}	22.35	17.0	18.1	14.29	11.5	7.02	12.02
iso-C _{16:0}	7.57	6.0	22.6	1.39	3.0	1.44	5.02
iso-C _{17:0}	—	—	—	1.09	—	—	1.37
anteiso-C _{15:0}	48.63	53.6	44.0	60.06	58.6	54.46	55.04
anteiso-C _{17:0}	3.45	7.0	3.1	5.44	41.0	10.69	8.77

it was concluded that strain H17^T showed peptidoglycan type A4 α , based on L-Lys-D-Asp.

Whole-cell sugars were detected with galactose present in large amounts, with smaller amounts of xylose and traces of mannose. This profile was different from that found for other species of the genus *Isoptericola*. Strain H17^T contained diphosphatidylglycerol, phosphatidylglycerol, phospholipids, phosphatidylinositol, phosphoglycolipid, one unidentified glycolipid and one unidentified lipid (see Fig. S2). The fatty acid profile (>0.5% of total fatty acids) of strain H17^T comprised branched fatty acids anteiso-C_{15:0} (48.63%), iso-C_{15:0} (22.35%), iso-C_{16:0} (7.57%), anteiso-C_{17:0} (3.45%), iso-C_{14:0} (2.13%) and iso-C_{17:0} (0.52%) and straight-chain fatty acids C_{16:0} (8.32%), C_{14:0} (5.97%) and C_{15:0} (0.91%) (Table 2). The menaquinone was MK9(H₄). Mycolic acids were not detected.

For analysis of the 16S rRNA gene sequence, bacterial DNA was extracted using a Qiagen Genomic DNA kit. The 16S rRNA gene was PCR-amplified using universal primers according to the methods of Timke *et al.* (2005) and directly sequenced on a sequencer (ABI 3730; Invitrogen). Phylogenetic analysis was performed using the MEGA (version 4.0) software after multiple alignments of data using

CLUSTAL_X. Distances were calculated according to the distance options with Kimura's two-parameter model and clustering with the neighbour-joining, minimum-evolution and the unweighted pair group method with arithmetic means (UPGMA) algorithms. Bootstrap values were determined based on 1000 replications. The neighbour-joining tree is shown in Fig. 1. The minimum-evolution and UPGMA trees are available as Figs S3 and S4. The DNA G + C content of strain H17^T was determined by the thermal denaturation method (Marmur & Doty, 1962) using *Escherichia coli* K-12 as a reference. DNA-DNA hybridization was carried out as described by De Ley *et al.* (1970) using a UV/VIS spectrophotometer (UV1201; Rayleigh).

A total of 1404 bp of the 16S rRNA gene sequence was sequenced. The sequence was subjected to similarity searches by using the sequence matching tool of the NCBI BLAST program (<http://www.ncbi.nlm.nih.gov>), Ribosomal Database Project II (<http://rdp.cme.msu.edu/>) and EzTaxon (<http://www.eztaxon.org/>; Chun *et al.* 2007). Comparative 16S rRNA gene sequence analysis showed that strain H17^T was most closely related to members of the genus *Isoptericola*. The 16S rRNA gene sequence of strain H17^T showed similarities of 99.6%, 98.4%, 98.2%, 98.1%, 98.0% and 97.8% with those of *I. variabilis* MX5^T, *I. hypogeus* HKI

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain H17^T, species of the genus *Isoptericola* and some other related taxa. Numbers at nodes are bootstrap values based on 1000 resamplings; only values >50% are given. *Arthrobacter globiformis* DSM 20124^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

0342^T, *I. jiangsuensis* CLG^T, *I. dokdonensis* DS-3^T, *I. halotolerans* YIM 70177^T and *I. chiayiensis* 06182M-1^T, respectively. The phylogenetic tree based on 16S rRNA gene sequences of strain H17^T and recognized species of the genus *Isoptericola* and other related species is shown in Fig. 1. In the phylogenetic tree based on the neighbour-joining algorithm, strain H17^T and *I. variabilis* MX5^T formed an independent cluster with a bootstrap value of 96 % (Fig. 1).

The G + C content of strain H17^T was 72.4 mol%, which was close to that found for other species of the genus *Isoptericola*. DNA–DNA hybridization studies showed relatively low relatedness values with *I. variabilis* MX5^T (45.6 %), *I. hypogaeus* HKI 0342^T (37.9 %), *I. jiangsuensis* CLG^T (33.4 %), *I. dokdonensis* DS-3^T (33.4 %), *I. halotolerans* YIM 70177^T (40.9 %) and *I. chiayiensis* 06182M-1^T (15.2 %). All of the values were significantly lower than 70 %, the threshold value recommended for the assignment of genomic species (Wayne *et al.*, 1987). These results indicated that strain H17^T represents a novel species of the genus *Isoptericola*, for which the name *Isoptericola nanjingensis* sp. nov. is proposed.

Description of *Isoptericola nanjingensis* sp. nov.

Isoptericola nanjingensis (nan.jing.en'sis. N.L. masc. adj. *nanjingensis* referring to Nanjing, Jiangsu Province, PR China, where the organism was first isolated).

Gram-positive-staining, aerobic, non-motile, non-spore-forming, coccus- or rod-shaped bacterium. Colonies are pale yellow, circular, convex, smooth and 1.0–2.0 mm in diameter after 48 h incubation at 30 °C. The optimum temperature, pH and NaCl concentration for growth are 30 °C, 7.0 and 3 % (w/v), respectively. In addition to the characteristics presented in Table 1, H₂S is not produced and the type strain gives a negative result for the Voges–Proskauer test. The cell-wall peptidoglycan type is L-Lys–D-Asp. The whole-cell sugars are galactose, xylose and mannose. The major fatty acids (>10 % of total) are anteiso-C_{15:0} and iso-C_{15:0}. The total polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phospholipids, phosphatidylinositol, phosphoglycolipid, one unidentified glycolipid and one unidentified lipid. The menaquinone is MK9(H₄) (see Fig. S5). Mycolic acids are not detected.

The type strain, H17^T (=DSM 24300^T=CCTCC AB 2011005^T), was isolated from soil in Nanjing, Jiangsu, PR China. The DNA G + C content of the type strain is 72.4 mol%.

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