Andreprevotia chitinilytica gen. nov., sp. nov., isolated from forest soil from Halla Mountain, Jeju Island, Korea

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A motile, Gram-negative, rod-shaped bacterium, designated strain JS11-7^T, was isolated from forest soil from Halla Mountain, Jeju Island, Korea. It grew optimally at 28 °C, pH 6–7 and in the presence of 0–2 % (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain JS11-7^T belongs to the family *Neisseriaceae*, with *Silvimonas terrae* as its closest relative (95.0 % similarity). It contained Q-8 as the predominant quinone and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω6c), C_{16:0} and C_{18:1}ω7c as the major fatty acids. The DNA G+C content was 62 mol%. On the basis of phenotypic and genotypic characteristics and the results of 16S rRNA gene sequence analysis, strain JS11-7^T represents a novel genus and species, for which the name *Andreprevotia chitinilytica* gen. nov., sp. nov. is proposed. The type species is *Andreprevotia chitinilytica* and the type strain is JS11-7^T (=KACC 11608^T=DSM 18519^T).

The soil of Jeju Island, located in southern Korea, is largely derived from ash from volcanic eruptions. Consequently, the microbial habitats of these soil environments are thought to differ from those of the Korea mainland. In the course of a study on the microbial community in forest soil from Jeju, we collected a soil sample from Halla Mountain on Jeju Island, Korea.

A 1 g soil sample was suspended in 9 ml 0.85 % NaCl (w/v) and mixed in a shaker for 30 min. After serial dilution, the aliquots of the serial diluents were spread on R2A agar plates (Reasoner & Geldreich, 1985) and incubated at 28 °C for 5 days. Isolate JS11-7^T was cultured routinely and maintained on R2A agar at 28 °C. It also grew on nutrient agar (Difco) and tryptic soy agar (TSA; Difco). However, no growth was observed on MacConkey agar (Difco).

The genomic DNA was isolated by using the method of Ausubel *et al.* (1987) except that the lysates were extracted

twice with chloroform to remove residual phenol. The 16S rRNA gene sequence of strain JS11-7^T was analysed as described by Kwon *et al.* (2003). The sequences obtained were compared with reference 16S rRNA gene sequences available from the GenBank and EMBL databases. A phylogenetic analysis was carried out using the MEGA3 program (Kumar *et al.*, 2004), after multiple alignments of the data had been obtained using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic dendrograms were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods, with bootstrap values based on 1000 replications. Analyses based on the 16S rRNA gene sequence (1407 bases of strain JS11-7^T) revealed that strain JS11-7^T had the highest level of sequence similarity (95.0 %) with respect to *Silvimonas terrae* KM-45^T (accession no. AB194302). On the other hand, all other species with validly published names showed low levels of sequence similarity (<92.2 %). These sequence similarity data are consistent with the phylogenetic dendrogram (Fig. 1), in which strain JS11-7^T clusters with *S. terrae* KM-45^T (Yang *et al.*, 2005) with 100 % bootstrap support. The genera *Formivibrio citricus* (Tanaka *et al.*, 1991), *Chitinibacter tainanensis* (Chern *et al.*, 2004) and *Iodobacter fluviatilis* (Logan, 1989) are also shown to be closely related to strain

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JS11-7^T is DQ836355.

A transmission electron micrograph of strain JS11-7^T and a list of the cellular fatty acids present in JS11-7^T and related species are available as supplementary data with the online version of this paper.

Fig. 1. Neighbour-joining phylogenetic tree, derived from 16S rRNA gene sequences, showing the position of strain JS11-7^T. Numbers at the nodes indicate levels of bootstrap support (based on 1000 resampled datasets). Bootstrap values below 50 % are not shown. Bar, 0.01 substitutions per site.

JS11-7^T. The monophyletic clade that includes these five taxa was also supported by the maximum-parsimony analysis.

The G+C content of the DNA of strain JS11-7^T was determined as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18-S; Supelco), and was found to be 62 mol%.

For the chemotaxonomic analysis, cells were grown for 48 h growth at 28 °C on TSA medium. A fatty acid methyl ester analysis was performed according to the standard protocol of the Microbial Identification System (MIDI; Microbial ID). Isoprenoid quinones were analysed by HPLC as described previously (Groth *et al.*, 1996). Strain JS11-7^T contained summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 39.5 %), C_{16:0} (31.1 %) and C_{18:1}ω7c (15.8 %) as the major fatty acids (see supplementary Table S1, available with the online version of this paper). The predominant isoprenoid quinone was Q-8.

To investigate the basic physiological and biochemical characteristics, we used the methods of Smibert & Krieg (1994) for the following tests: the Gram reaction, oxidase and catalase reactions, the deamination of phenylalanine, the Voges–Proskauer test and the hydrolysis of CM-cellulose, casein, chitin, DNA, pectin, starch, tyrosine and Tween 80. The temperature range (5–45 °C, using increments of 5 °C), pH range (4–10, using increments of 1 pH unit) and NaCl requirement (0, 1, 2, 3 and 5 % NaCl, w/v) were determined using R2A medium. For other classical and phenotypic tests, API 20NE, API ID 32GN and API ZYM test kits were also used according to the recommendations of the manufacturer (bioMérieux). The

API ZYM tests were read after 4 h incubation at 37 °C; the other API tests were read after 48 h at 28 °C. Cell morphology was observed by using transmission electron microscopy and phase-contrast microscopy (Axio; Zeiss). For transmission electron microscopy, cells were grown on TSA and suspended in physiological saline solution. A small drop of suspension was placed on a carbon-coated copper grid and the cells were negatively stained with 0.5 % uranyl acetate for observation under the electron microscope (model 912AB; LEO).

The cells of strain JS11-7^T were straight rods, 0.7–0.9 µm in diameter and 2.0–3.0 µm in length (see supplementary Fig. S1, available with the online version of this paper). The phenotypic properties of JS11-7^T are listed in Table 1 and in the genus and species descriptions.

Strain JS11-7^T was clearly different from its close neighbours, *S. terrae*, *F. citricus*, *C. tainanensis* and *I. fluviatilis*, as determined by comparative analyses of 16S rRNA gene sequences. *F. citricus* could be easily differentiated from strain JS11-7^T and other closely related genera in that it was strictly anaerobic, had the ability to hydrolyse chitin, and contained a relatively large amount (9.5 %) of C_{16:0} 3-OH (Tanaka *et al.*, 1991; Chern *et al.*, 2004). Strain JS11-7^T differs from *S. terrae*, the closest phylogenetic relative, in terms of several phenotypic properties (the former is unable to grow anaerobically, to reduce nitrate, to hydrolyse aesculin and to grow at 40 °C, but is able to hydrolyse gelatin) and in terms of fatty acid composition [the former lacks C_{19:0}ω8c cyclo and summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} I), contains small amounts of C_{14:0} and C_{17:0} cyclo, and a large amount of summed feature 3] (Tables 1 and S1).

Table 1. Differential characteristics for JS11-7^T with respect to its closest phylogenetic neighbours

Strains: 1, JS11-7^T; 2, *S. terrae* KCTC 12358^T (Yang *et al.*, 2005; this study); 3, *C. tainanensis* DSM 15459^T (Chern *et al.*, 2004; this study); 4, *I. fluvialis* DSM 3764^T (Logan, 1989; this study). All of the strains are negative for indole production, arginine dihydrolase and urease. All assimilate D-glucose, N-acetylglucosamine, potassium gluconate and D-ribose. None of the strains assimilates capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, inositol, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, D-melibiose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate or 4-hydroxybenzoic acid. All are positive for esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl- β -glucosaminidase. All are negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase. +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3	4
Anaerobic growth	–	+*	–†	+‡
Growth at 40 °C	–	+*	+†	–‡
Nitrate reduction	–	+	–	+
Glucose fermentation	+	+	–	–
Aesculin hydrolysis	–	+	–	–
Gelatin hydrolysis	+	–	–	+
Assimilation of:				
L-Arabinose	+	+	–	–
D-Mannose	(+)	+	–	+
D-Mannitol	+	+	–	–
D-Maltose	+	+	–	+
L-Rhamnose	+	+	–	–
D-Sucrose	–	+	–	–
Sodium acetate	(+)	(+)	–	+
Glycogen	+	(+)	–	(+)
L-Serine	–	–	+	+
Salicin	–	+	–	–
L-Fucose	–	+	–	–
3-Hydroxybutyric acid	+	(+)	–	(+)
L-Proline	(+)	(+)	–	+
Enzyme activities:				
β -Galactosidase	+	+	–	–
Alkaline phosphatase	+	+	–	+
Esterase lipase (C8)	–	+	–	+
α -Glucosidase	+	+	–	+
β -Glucosidase	–	+	–	–
DNA G + C content (mol%)	62	58*	56†	50–52‡

*Data from Yang *et al.* (2005).

†Data from Chern *et al.* (2004).

‡Data from Logan (1989).

On the basis of the results of the polyphasic analysis, strain JS11-7^T represents a novel genus and species, for which the name *Andreprevotia chitinilytica* gen. nov., sp. nov. is proposed.

Description of *Andreprevotia* gen. nov.

Andreprevotia (An.dre.pre.vo.ti'a. N.L. fem. n. *Andreprevotia* named after André Romain Prévot, the French microbiologist who first named the family *Neisseriaceae*).

Colonies are ivory-coloured, round and convex with clear margins on R2A medium. Cells are strictly aerobic, Gram-negative, motile with single polar or subpolar flagella and rod-shaped (0.7–0.9 $\times$ 2.0–3.0 μ m). Positive for oxidase activity, catalase activity and glucose fermentation. Negative for nitrate reduction, Voges–Proskauer reaction and phenylalanine deamination. Major isoprenoid quinone is Q-8. Fatty acids mainly comprise summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c), C_{16:0} and C_{18:1} ω 7c. The DNA G + C content of the type strain of the type species is 62 mol%. 16S rRNA gene sequence analysis places *Andreprevotia* in the family *Neisseriaceae*. The type species is *Andreprevotia chitinilytica*.

Description of *Andreprevotia chitinilytica* sp. nov.

Andreprevotia chitinilytica (chi.ti.ni.ly'ti.ca. N.L. n. *chitinum* chitin; N.L. adj. *lyticus* dissolving; N.L. fem. adj. *chitinilytica* chitin-dissolving).

Growth is observed at 5–35 °C (optimum, 28 °C), at pH 5.0–8.0 (optimum, pH 6.0–7.0) and in medium containing 0–2 % (w/v) NaCl. Degrades Tween 80. Does not degrade aesculin, casein, pectin or tyrosine. According to the API 20NE and API ID 32GN test strips, assimilates D-glucose, L-arabinose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, L-rhamnose, D-ribose, glycogen and 3-hydroxybutyric acid. Shows weak assimilation of D-mannose, sodium acetate and L-proline. Does not assimilate capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, inositol, D-sucrose, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, D-melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate or 4-hydroxybenzoic acid. With the API ZYM test strips, positive results are obtained for alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and N-acetyl- β -glucosaminidase. Negative for esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase.

The type strain, JS11-7^T (= KACC 11608^T = DSM 18519^T), was isolated from forest soil from Halla Mountain, Jeju Island, Republic of Korea.

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