Jeongeupia naejangsanensis gen. nov., sp. nov., a cellulose-degrading bacterium isolated from forest soil from Naejang Mountain in Korea

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A Gram-stain-negative, motile, rod-shaped, cellulose-degrading bacterial strain, BIO-TAS4-2^T, which belongs to the *Betaproteobacteria*, was isolated from forest soil from Naejang Mountain, Korea, and its taxonomic position was investigated by using a polyphasic study. Strain BIO-TAS4-2^T grew optimally at pH 7.0–8.0, at 30 °C and in the presence of 0–1.0 % (w/v) NaCl. Phylogenetic trees based on 16S rRNA gene sequences showed that strain BIO-TAS4-2^T clustered with members of the genera *Andreprevotia*, *Silvimonas* and *Deefgea* of the family *Neisseriaceae*, with which it exhibited 16S rRNA gene sequence similarities of 93.5–94.2 %. Strain BIO-TAS4-2^T contained Q-8 as the predominant ubiquinone and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and C_{16:0} as the major fatty acids. The DNA G + C content was 63.8 mol%. Strain BIO-TAS4-2^T could be differentiated from members of phylogenetically related genera by differences in fatty acid composition, DNA G + C content and some phenotypic properties. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain BIO-TAS4-2^T is considered to represent a novel species in a new genus, for which the name *Jeongeupia naejangsanensis* gen. nov., sp. nov. is proposed, with BIO-TAS4-2^T (=KCTC 22633^T=CCUG 57610^T) as the type strain.

During the screening of cellulose-degrading micro-organisms from forest soil collected from Naejang Mountain in Korea, many bacterial strains have been isolated and characterized taxonomically. One of these isolates, BIO-TAS4-2^T, which formed a distinct phylogenetic lineage within the *Betaproteobacteria*, is the subject of this study. The aim of the present work was to determine the taxonomic position of strain BIO-TAS4-2^T by using a polyphasic characterization that included determination of the phenotypic and chemotaxonomic properties, as well as a phylogenetic investigation based on 16S rRNA gene sequences.

Bacterial strains were isolated from a soil sample collected from Naejang Mountain, Korea. The soil sample (1 g) was added to PBS buffer and serially diluted. The diluted suspension was spread onto solid Luria–Bertani (BD) medium, adjusted to initial pH 7.0, containing 0.2 % (w/v) Azo-CM-cellulose (Megazyme). The culture was incubated at 37 °C for 3–5 days. Cellulolytic micro-organisms were isolated if clear zones around colonies due to degradation

of Azo-CM-cellulose were observed. Of the isolates, strain BIO-TAS4-2^T, which showed an excellent ability to form a clear zone, was selected and used for further study. The type strains of three closely related species were used as reference strains for phenotypic characterization and fatty acid analysis: *Andreprevotia chitinilytica* DSM 18519^T and *Deefgea rivuli* DSM 18356^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; and *Silvimonas terrae* KCTC 12358^T was obtained from the Korean Collection for Type Cultures, Taejeon, Korea. To investigate morphological, physiological and biochemical characteristics, strain BIO-TAS4-2^T was cultivated routinely on trypticase soy agar (TSA; Difco) at 30 °C. Cell morphology was examined by light microscopy (Nikon E600) using cells from exponentially growing cultures. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 40, 45, 50 and 55 °C) was measured in trypticase soy broth (TSB; Difco). The pH range for growth was determined in nutrient broth (Difco) adjusted, prior to sterilization, to various pH values (pH 4.5–10.5 at intervals of 0.5) by

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using sodium acetate/acetic acid or Na_2CO_3 buffers. Growth in various NaCl concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 %, w/v) was investigated using TSB prepared according to the formula of the Difco medium except that NaCl was excluded. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were examined as described by Lányi (1987). Susceptibility to antibiotics was tested by placing antibiotic-impregnated discs on TSA plates that were seeded with suspensions of the test strain. Antibiotics tested were polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) and carbenicillin (100 µg). Utilization of various substrates, enzyme activities, and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50 CH and API ZYM systems (bioMérieux). Cells were suspended in AUX medium according to the manufacturer's instructions to inoculate the API 50 CH system.

Cell biomass of strain BIO-TAS4-2^T for DNA extraction and for isoprenoid quinone analysis was obtained from cultures grown in TSB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Alignment of sequences was carried out with CLUSTAL W software (Thompson *et al.*, 1994). Gaps at the 5' and 3' ends of the alignment were omitted from further analysis. Phylogenetic trees were inferred by using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods implemented within the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated by using the algorithm of Jukes & Cantor (1969) with the program DNADIST. The stability of relationships was assessed by bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package. Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A

(250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain BIO-TAS4-2^T, *A. chitinilytica* DSM 18519^T, *D. rivuli* DSM 18356^T and *S. terrae* KCTC 12358^T was harvested from TSA plates after incubation for 3 days at 30 °C. Fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain BIO-TAS4-2^T are given in the genus and species descriptions (see below) or are shown in Table 1. The almost complete 16S rRNA gene sequence of strain BIO-TAS4-2^T determined in this study comprised 1490 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree based on the neighbour-joining algorithm, strain BIO-TAS4-2^T formed a cluster with members of the genera *Andreprevotia*, *Silvimonas* and *Deefgea*, which belong to the family *Neisseriaceae* (Fig. 1). This topology was also found in trees constructed using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain BIO-TAS4-2^T exhibited 16S rRNA gene sequence similarities of 94.2, 93.5 and 93.6 % to the type strains of *A. chitinilytica*, *S. terrae* and *D. rivuli*, respectively, and of less than 92.2 % to other species used in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain BIO-TAS4-2^T was ubiquinone-8 (Q-8). The cellular fatty acid profile of strain BIO-TAS4-2^T is shown in Table 2, together with those of members of the genera *Andreprevotia*, *Silvimonas* and *Deefgea* also analysed in this study. Strain BIO-TAS4-2^T had large amounts of unsaturated, straight-chain and hydroxy fatty acids; the major components (>10 % of total fatty acids) were summed feature 3 ($\text{C}_{16:1}\omega 7c$ and/or iso- $\text{C}_{15:0}$ 2-OH) and $\text{C}_{16:0}$. The DNA G + C content of strain BIO-TAS4-2^T was 63.8 mol%.

Phylogenetic analyses based on 16S rRNA gene sequences showed that strain BIO-TAS4-2^T does not fall within the radiation encompassed by a recognized genus, but forms an independent evolutionary lineage within the family *Neisseriaceae* belonging to the *Betaproteobacteria* (Fig. 1). The 16S rRNA gene sequence similarity values between strain BIO-TAS4-2^T and other genera were relatively low (<94.2 %). The predominant ubiquinone type (Q-8) of strain BIO-TAS4-2^T was the same as that of members of the phylogenetically related genera *Andreprevotia*, *Silvimonas* and *Deefgea* (Yang *et al.*, 2005; Stackebrandt *et al.*, 2007; Weon *et al.*, 2007). When cultivated under identical conditions, the fatty acid profile of strain BIO-TAS4-2^T differed from those of the phylogenetically related genera (Table 2). Strain BIO-TAS4-2^T could be differentiated from members of the genera *Andreprevotia* and *Silvimonas* by differences in phenotypic properties, includ-

Table 1. Differential phenotypic characteristics of *Jeongeupia naejangsanensis* BIO-TAS4-2^T and some phylogenetically related taxa

Taxa: 1, strain BIO-TAS4-2^T (*Jeongeupia naejangsanensis* gen. nov., sp. nov.); 2, *A. chitinilytica* (data from Weon *et al.*, 2007; and this study); 3, *S. terrae* (Weon *et al.*, 2007; and this study); 4, *D. rivuli* (Stackebrandt *et al.*, 2007; and this study). +, Positive reaction; –, negative reaction; w, weakly positive reaction; NG, no growth. All taxa are positive for motility, catalase, oxidase and *N*-acetyl- β -glucosaminidase activities, and utilization of ribose, fructose and gluconate. All taxa are negative for Gram-staining, utilization of glycerol, erythritol, L-xylose, methyl β -D-xyloside, sorbose, dulcitol, inositol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, amygdalin, arbutin, lactose, melibiose, inulin, melezitose, raffinose, D-tagatose, D-fucose, 2-ketogluconate, 5-ketogluconate and phenylacetate, and lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase activities.

Characteristic	1	2	3	4
Anaerobic growth	+	–	+	+
Growth at 40 °C	+	–	+	–
Nitrate reduction	+	–	+	+
Glucose fermentation	+	+	+	–
Hydrolysis of:				
Aesculin	–	–*	+	–*
Casein	–	–*	–*	+
Gelatin	–	+	–	–
Starch	–	+	+	–*
Tween 80	–	+	+	NG*
Utilization of:*				
D-Arabinose	–	–	+	–
L-Arabinose	–	+	+	–
D-Xylose	–	+	+	–
Adonitol	+	–	–	–
Galactose	–	+	+	–
Glucose	+	+	–	+
Mannose	+	+	+	–
Rhamnose	–	+	+	–
D-Mannitol	–	+	+	–
N-Acetylglucosamine	+	+	+	–
Aesculin	–	–	+	–
Salicin	–	–	+	–
Cellobiose	–	–	+	–
Maltose	–	+	+	–
Sucrose	–	–	+	+
Trehalose	–	–	+	–
Starch	–	+	+	–
Glycogen	–	+	+	–
Xylitol	+	–	–	–
Gentiobiose	–	+	+	–
Turanose	–	–	+	–
D-Lyxose	+	–	+	–
L-Fucose	–	–	+	–
D-Arabitol	–	+	+	–
L-Arabitol	+	–	–	–
Adipate	–	–	+	–

Table 1. cont.

Characteristic	1	2	3	4
Caprate	+	–	–	–
Malate	+	+	+	–
Citrate	+	–	–	–
Enzyme activity (by API ZYM):*				
Alkaline phosphatase	–	–	w	–
Lipase (C4)	w	–	–	–
Esterase lipase (C8)	w	w	w	–
Leucine arylamidase	+	w	w	w
Acid phosphatase	+	+	+	–
Naphthol-AS-BI-phosphohydrolase	–	–	+	w
α -Glucosidase	–	+	+	–
DNA G + C content (mol%)	63.8	62	58	49

*Data for reference species determined in this study are those of the type strains.

ing anaerobic growth, nitrate reduction, hydrolysis of some substrates and utilization of substrates, as shown in Table 1, as well as by differences in fatty acid composition, particularly C_{18:1} ω 7c and cyclo-C_{17:0}, as shown in Table 2. Strain BIO-TAS4-2^T also differed from *D. rivuli* in its DNA G + C content and by differences in physiological properties (Table 1). Therefore, on the basis of the phylogenetic data and differential chemotaxonomic and phenotypic properties, strain BIO-TAS4-2^T is considered to represent a novel species in a new genus within the *Betaproteobacteria*, for which the name *Jeongeupia naejangsanensis* gen. nov., sp. nov. is proposed.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Jeongeupia naejangsanensis* BIO-TAS4-2^T and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. *E. coli* ATCC 11775^T was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid contents (%) of *Jeongeupia naejangsensis* BIO-TAS4-2^T and some phylogenetically related strains

Strains: 1, BIO-TAS4-2^T (*Jeongeupia naejangsensis* gen. nov., sp. nov.); 2, *A. chitinilytica* DSM 18519^T; 3, *S. terrae* KCTC 12358^T; 4, *D. rivuli* DSM 18356^T. All data are from this study. Fatty acids that represent <0.5 % in all strains were omitted. —, Not detected.

Fatty acid	1	2	3	4
Straight-chain				
C _{12:0}	—	2.8	4.1	2.9
C _{14:0}	8.3	0.7	8.8	2.5
C _{15:0}	4.0	0.7	1.8	5.0
C _{16:0}	25.5	31.2	29.1	17.4
C _{18:0}	—	0.6	0.1	0.2
Unsaturated				
C _{14:1} ω5c	0.4	0.3	1.1	0.2
C _{15:1} ω6c	1.2	0.6	0.5	3.8
C _{15:1} ω8c	0.1	—	0.1	0.6
C _{16:1} ω5c	—	0.4	1.0	0.3
C _{17:1} ω6c	6.3	—	—	0.4
C _{17:1} ω8c	—	—	—	1.1
C _{17:1} ω9c	0.9	—	—	—
C _{18:1} ω7c	—	15.8	12.7	2.9
Cyclo				
C _{17:0}	—	3.5	21.7	—
C _{19:0} ω8c	—	0.2	1.1	—
Hydroxy				
C _{10:0} 3-OH	—	1.6	0.6	—
C _{12:0} 3-OH	5.3	2.4	0.1	2.4
C _{18:1} 2-OH	—	—	0.7	—
Summed features*				
2	—	—	4.8	0.4
3	47.1	38.2	10.8	56.6

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2 contains C_{14:0} 3-OH and/or iso-C_{16:1}. Summed feature 3 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

Description of *Jeongeupia* gen. nov.

Jeongeupia (Je.ong.eup.i'a. N.L. fem. n. *Jeongeupia* named after Jeongeup, a Korean city, the location of Naejang Mountain).

Cells are Gram-stain-negative and rod-shaped. Facultatively anaerobic. Catalase- and oxidase-positive. The predominant ubiquinone is Q-8. The major fatty acids (>10 % of total fatty acids) are summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and C_{16:0}. The type species is *Jeongeupia naejangsensis*.

Description of *Jeongeupia naejangsensis* sp. nov.

Jeongeupia naejangsensis (nae.jang.san.en'sis. N.L. fem. adj. *naejangsensis* pertaining to Naejangsan, the Korean

name of Naejang Mountain, from where the type strain was isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.3–0.7 × 0.7–2.5 µm. Motile by means of a single polar flagellum. Colonies on TSA are circular to slightly irregular, flat, smooth, glistening, greyish-yellow in colour and 3.0–5.0 mm in diameter after incubation for 3 days at 30 °C. Optimal temperature for growth is 30 °C. Growth occurs at 10 and 50 °C, but not at 4 or 55 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.0, but not at pH 4.5. Growth occurs in the presence of 0–3.0 % (w/v) NaCl, with optimum growth in the presence of 0–1.0 % (w/v) NaCl. Anaerobic growth occurs on TSA and on TSA supplemented with nitrate. Cellulose is degraded. Aesculin, casein, gelatin, hypoxanthine, starch, L-tyrosine, urea, xanthine, and Tweens 20, 40, 60 and 80 are not hydrolysed. Nitrate is reduced to nitrite. Indole and H₂S are not produced. Glucose is fermented. The following substrates are utilized as carbon and energy sources: N-acetylglucosamine, adonitol, L-arabitol, caprate, citrate, fructose, gluconate, glucose, D-lyxose, malate, mannose, ribose and xylitol. The following substrates are not utilized as carbon and energy sources: adipate, aesculin, amygdalin, D-arabinose, L-arabinose, D-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, L-fucose, galactose, gentiobiose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, maltose, D-mannitol, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, phenylacetate, raffinose, rhamnose, salicin, sorbitol, sorbose, starch, sucrose, D-tagatose, trehalose, turanose, D-xylose and L-xylose. In assays with the API ZYM system: positive for leucine arylamidase, acid phosphatase and N-acetyl-β-glucosaminidase; weakly positive for esterase (C4) and esterase lipase (C8); and negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. Susceptible to chloramphenicol, gentamicin, kanamycin, neomycin, polymyxin B, streptomycin and tetracycline, but not to ampicillin, carbenicillin, cephalothin, lincomycin, novobiocin, oleandomycin or penicillin G.

The type strain is BIO-TAS4-2^T (=KCTC 22633^T=CCUG 57610^T), isolated from soil from Naejang Mountain, Jeongeup city, Korea. The DNA G+C content of the type strain is 63.8 mol% (determined by HPLC).

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