Solirubrobacter ginsenosidimutans sp. nov., isolated from soil of a ginseng field

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A Gram-reaction-positive, non-motile, non-spore-forming, aerobic rod, designated BXN5-15^T, was isolated from the soil of a ginseng field on Baekdu Mountain in China. Strain BXN5-15^T grew optimally at 30 °C and pH 6.0–7.0 in the absence of NaCl on R2A agar. Strain BXN5-15^T displayed β -glucosidase activity, which allowed it to transform ginsenoside Rb₁ (one of the dominant active components of ginseng) via Rd to minor ginsenoside F₂. On the basis of 16S rRNA gene sequence analysis, strain BXN5-15^T was shown to belong to the genus *Solirubrobacter*. The closest phylogenetic relatives were *Solirubrobacter soli* Gsoil 355^T (98.4 % 16S rRNA gene sequence similarity) and *Solirubrobacter pauli* B33D1^T (96.4 %). Lower sequence similarities (<96.0 %) were found with all of the other recognized members of the order *Solirubrobacterales*. The predominant quinone was MK-7(H₄). The major fatty acids (>10 %) were C_{18:1}ω9c, iso-C_{16:0} and C_{18:3}ω6,9,12c. The G+C content of the genomic DNA was 70.6 mol%. DNA–DNA relatedness between strain BXN5-15^T and *S. soli* KCTC 12628^T was 23.3 %. On the basis of genotypic, phenotypic and chemotaxonomic data, strain BXN5-15^T represents a novel species within the genus *Solirubrobacter*, for which the name *Solirubrobacter ginsenosidimutans* sp. nov. is proposed. The type strain is BXN5-15^T (=KACC 20671^T =LMG 24459^T).

The genus *Solirubrobacter* was established by Singleton *et al.* (2003) with the description of a single species, *Solirubrobacter pauli*, isolated from a burrow of the earthworm *Lumbricus rubellus* in agricultural soil in Georgia, USA (Furlong *et al.*, 2002). Later, the species *Solirubrobacter soli* was isolated from soil of a ginseng field in South Korea (Kim *et al.*, 2007). Members of the genus *Solirubrobacter* are characterized as Gram-positive, non-spore-forming, aerobic and catalase-positive rods, with C_{18:1}ω9c and iso-C_{16:0} as

the major fatty acids and DNA G+C contents of 71.5–71.8 mol%.

During the course of a study on the cultivable aerobic bacterial community in soil of a ginseng field on Baekdu Mountain in China, a large number of novel bacterial strains were isolated. One of these isolates, strain BXN5-15^T, appeared to be a member of genus *Solirubrobacter* and converted ginsenoside Rb₁ {3-O- β -D-glucopyranosyl-(1-2)- β -D-glucopyranosyl]-20-O- β -D-glucopyranosyl-(1-6)- β -D-glucopyranosyl]-20(S)-protopanaxadiol} via Rd {3-O- β -D-glucopyranosyl-(1-2)- β -D-glucopyranosyl]-20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol} to minor ginsenoside F₂ [3-O-(β -D-glucopyranosyl)-20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol].

For isolation of strain BXN5-15^T, a soil sample was thoroughly suspended and serially diluted in 50 mM phosphate buffer (pH 7.0) and spread on modified xylan-nutrient agar comprising (l⁻¹) 0.02 g Tryptone, 0.02 g yeast extract, 0.02 g malt extract, 0.02 g beef extract, 0.02 g Casamino acid, 0.02 g Soytone, 1.0 g xylan, 0.1 g

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Abbreviations: Rb₁, 3-O- β -D-glucopyranosyl-(1-2)- β -D-glucopyranosyl]-20-O- β -D-glucopyranosyl-(1-6)- β -D-glucopyranosyl]-20(S)-protopanaxadiol; Rd, 3-O- β -D-glucopyranosyl-(1-2)- β -D-glucopyranosyl]-20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol; F₂, 3-O-(β -D-glucopyranosyl)-20-O- β -D-glucopyranosyl-20(S)-protopanaxadiol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BXN5-15^T is EU332825.

A supplementary table and a supplementary figure are available with the online version of this paper.

sodium pyruvate, 0.3 g K_2HPO_4 , 0.05 g $MgSO_4$, 0.05 g $CaCl_2$, 15 g agar; pH 7.0). The plates were incubated at 30 °C for 1 month. Strain BXN5-15^T was cultured routinely on R2A agar (Difco) at 25 °C and preserved in 20 % (w/v) glycerol at -70 °C.

The genomic DNA of strain BXN5-15^T was extracted using a commercial genomic DNA-extraction kit (Solgent). The 16S rRNA gene was amplified using the universal bacterial primer pair 9F and 1512R and the purified PCR products were sequenced by Solgent (Ten *et al.*, 2008). An almost-complete sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR). 16S rRNA gene sequences of related taxa were obtained from the GenBank database and the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Multiple alignments were performed using CLUSTAL X (Thompson *et al.*, 1997) and gaps were edited using BioEdit (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using MEGA version 4 (Kumar *et al.*, 2008) and bootstrap values were calculated from 1000 replications (Felsenstein, 1985).

The 16S rRNA gene sequence of strain BXN5-15^T determined in this study was a continuous stretch of 1490 bp (base positions 24–1507 with respect to the *Escherichia coli* numbering system). Sequence similarity calculations indicated that the closest relatives of the isolate were *S. soli* Gsoil 355^T (98.4 % 16S rRNA gene sequence similarity) and *S. pauli* B33D1^T (96.4 %). Sequence similarities <96.0 % were found with all other recognized members of the order *Solirubrobacterales*. The relationship between strain BXN5-15^T and members of the order *Solirubrobacterales* was also evident in the phylogenetic tree (Fig. 1). On the basis of the phylogenetic inference, *S. soli* KCTC 12628^T and *S. pauli* KCTC 9974^T were used as reference strains for most phenotypic tests and for DNA–DNA hybridization studies.

The Gram-reaction was performed by the non-staining method as described by Buck (1982). Morphology and motility of cells grown for 3 days at 30 °C on R2A agar were determined by using light microscopy (E600; Nikon) and transmission electron microscopy (negative staining with 1 %, w/v, phosphotungstic acid; CM-20; Philips).

Catalase activity was assessed by bubble production in 3 % (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Carbon-source utilization and enzyme activities were tested using API 20 NE, API ID 32 GN and API ZYM kits (bioMérieux), according to the manufacturer's instructions. Growth at 4, 10, 18, 25, 30, 37, 42 and 45 °C was assessed after 7 days on R2A agar. Growth at pH 5.0–10.0 (at intervals of 0.5 pH units) was assessed after 7 days in R2A broth with the pH adjusted using the following buffer system (final concentration 50 mM): pH 5.0–5.5, acetate buffer; pH 6.0–8.0, phosphate buffer; and pH 8.5–10.0, Tris buffer. Growth in 1–10 % (w/v) NaCl (at intervals of 1 % NaCl) was assessed after 7 days in R2A broth by monitoring OD₆₀₀. Anaerobic growth was tested in serum bottles by adding 1 g thioglycolate 1⁻¹ to R2A broth and substituting the gas phase with nitrogen gas. Growth was also evaluated on trypticase soy agar (TSA; BD) and MacConkey agar (BD) at 30 °C. Conversion of ginsenoside Rb₁ was assessed using TLC and HPLC as described by Kim *et al.* (2005).

Cells of strain BXN5-15^T were Gram-reaction-positive, strictly aerobic, non-spore-forming, non-motile, oxidase- and catalase-positive rods (Supplementary Fig. S1, available in IJSEM Online). β -Glucosidase activity was detected in strain BXN5-15^T; this activity was responsible for the gradual conversion of ginsenoside Rb₁ to compound F₂ (data not shown). The morphological, physiological and biochemical characteristics of strain BXN5-15^T are given in the species description and Table 1.

To measure the G + C content of the chromosomal DNA of strain BXN5-15^T, genomic DNA was extracted and purified as described by Moore & Dowhan (1995) and the G + C content was determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Cellular fatty acid compositions of the isolate and the reference strains were determined using cells grown on R2A agar for 3 days according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acid methyl esters were analysed by GC (6890; Hewlett Packard) and identified by Microbial Identification software (Sasser, 1990). For the determination of isoprenoid quinones, strain BXN5-15^T was grown in R2A broth for 5 days at 30 °C and harvested cells were lyophilized for 24 h. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1400 bp), showing the relationship of strain BXN5-15^T with related taxa. Bootstrap values (>70 %) based on 1000 replications are shown at branch nodes. Filled circles indicate corresponding nodes that were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.02 substitutions per nucleotide position.

Table 1. Differentiating characteristics of strain BXN5-15^T and type strains of species of the genus *Solirubrobacter*

Strains: 1, *Solirubrobacter ginsenosidimutans* sp. nov. BXN5-15^T; 2, *S. soli* KCTC 12628^T; 3, *S. pauli* KCTC 9974^T. Data were taken from this study unless otherwise indicated. All strains were Gram-reaction-positive rods and positive for catalase, esterase (C4), esterase lipase (C8), leucine arylamidase, α -glucosidase and β -glucosidase. All strains were negative for lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, α -fucosidase, α -galactosidase, β -glucuronidase, arginine dihydrolase, urease, production of indole, acid production from glucose and reduction of nitrate to nitrite. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3
Cell length (μ m)*	1.8–2.5	1.0–3.0	1.5
Growth temperature ($^{\circ}$ C)*			
Range	18–37	15–30*	19–38*
Optimum	30	25–30*	28–30*
Growth in 1% NaCl	–	+	–
API 20 NE			
Oxidase	+	w	–
Gelatin hydrolysis	–	+	–
Assimilation of:			
Glucose	–	+	w
Arabinose	+	+	w
Mannose	–	+	+
Mannitol	w	w	–
N-Acetylglucosamine	–	w	–
Maltose	–	+	–
Gluconate	+	w	+
Adipate	–	w	–
API ZYM			
Valine arylamidase	w	+	+
Naphthol-AS-BI-phosphohydrolase	–	–	+
N-Acetyl- β -glucosaminidase	–	+	–
α -Mannosidase	–	–	+
β -Galactosidase	+	–	–
DNA G+C content (mol%)	70.6	71.5*	71.8*

*Data were taken from Singleton *et al.* (2003) and Kim *et al.* (2007).

re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane/quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed by HPLC as described by Hiraishi *et al.* (1996). DNA–DNA hybridization was performed between strain BXN5-15^T and *S. soli* KCTC 12628^T using the method described by Ezaki *et al.* (1989) with photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed reciprocally with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the mean of the remaining three values was converted to obtain a DNA–DNA relatedness value.

The DNA G+C content of strain BXN5-15^T was 70.6 mol%, which is similar to those of *S. pauli* and *S.*

soli (Table 1). The respiratory quinone system of strain BXN5-15^T was MK-7(H₄), which is consistent with the quinone systems of members of the genus *Solirubrobacter* (Kim *et al.*, 2007). The fatty acid profiles of strain BXN5-15^T and the reference strains are shown in Supplementary Table S1. The major fatty acids of strain BXN5-15^T were C_{18:1} ω 9c (14.8%) and iso-C_{16:0} (12.9%), which were typical of the genus *Solirubrobacter*, and C_{18:3} ω 6,9,12c (11.1%), which was not found in *S. soli* KCTC 12628^T. Minor fatty acids (2–10%) were iso-C_{16:0} 3-OH (7.9%), C_{17:1} ω 6c (6.2%), iso-C_{19:0} (5.9%), iso-C_{20:0} (5.2%), C_{18:0} (5.1%), C_{16:0} (4.7%), C_{20:4} ω 6,9,12,15c (4.0%), iso-C_{18:0} (2.3%) and C_{14:0} (2.1%). Some minor qualitative and quantitative differences in fatty acid content were observed between strain BXN5-15^T and its closest relatives. DNA–DNA relatedness between strain BXN5-15^T and *S. soli* KCTC 12628^T was (mean $\pm$ SD) 23.3 $\pm$ 4.8% (reciprocal experiment, 20.8 $\pm$ 4.2%). These values are low enough to assign strain BXN5-15^T to a novel species of genus *Solirubrobacter* (Wayne *et al.*, 1987).

In summary, the characteristics of strain BXN5-15^T were consistent with descriptions of the genus *Solirubrobacter* with regard to morphological, biochemical and chemotaxonomic properties. However, the phylogenetic distances and the low level of DNA–DNA relatedness between strain BXN5-15^T and recognized members of the genus *Solirubrobacter* and the unique phenotypic characteristics (Table 1) warrant assignment of strain BXN5-15^T to the genus *Solirubrobacter* as a novel species, for which the name *Solirubrobacter ginsenosidimutans* sp. nov. is proposed.

Description of *Solirubrobacter ginsenosidimutans* sp. nov.

Solirubrobacter ginsenosidimutans (gin.se.no.si.di.mu'tans. N.L. n. *ginsenosidum* ginsenoside; L. part. adj. *mutans* transforming, converting; N.L. part. adj. *ginsenosidimutans* ginsenoside-converting).

Cells are Gram-reaction-positive, non-motile, non-spore-forming, aerobic rods (0.4–0.6 μ m wide, 1.8–2.5 μ m long after culture on R2A agar for 3 days). Colonies are smooth, circular, convex, yellow and 0.6–1.2 mm in diameter. Grows at 18–37 $^{\circ}$ C (optimum 30 $^{\circ}$ C), but not at 10 or 42 $^{\circ}$ C, at pH 6.0–7.0 (optimum pH 7.0) and in <1.0% (w/v) NaCl. Catalase- and oxidase-positive. Grows on nutrient agar and TSA agar, but not on MacConkey agar. Sole carbon sources (API 20 NE and API ID 32GN) and enzyme activities (API ZYM) are listed in Table 1. In addition, gluconate is utilized as a sole carbon source, but caprate, malate, citrate and phenylacetate are not. Does not reduce nitrate to nitrite or nitrogen gas. MK-7(H₄) is the predominant menaquinone. The major fatty acids (>7%) are C_{18:1} ω 9c, iso-C_{16:0}, C_{18:3} ω 6,9,12c and iso-C_{16:0} 3-OH.

The type strain, BXN5-15^T (=KACC 20671^T =LMG 24459^T), was isolated from soil of a ginseng field on Baekdu Mountain, China. The DNA G+C content of the type strain is 70.6 mol%.

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