Dyella koreensis sp. nov., a β -glucosidase-producing bacterium

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A bacterial strain (designated BB4^T), which has β -glucosidase activity, was isolated from soil around the roots of bamboo plants. Cells were Gram-negative, aerobic, non-motile and straight-rod-shaped. Phylogenetic analysis of 16S rRNA gene sequences revealed a clear affiliation with members of the family 'Xanthomonadaceae'. The 16S rRNA gene sequence of strain BB4^T showed the following sequence similarities: 97.7% to *Dyella japonica* XD53^T, 97.1% to *Frateuria aurantia* LMG 1558^T, 96.2% to *Fulvimonas soli* LMG 19981^T, 94.3% to *Rhodanobacter lindaniclasticus* RP5575^T and <90% to other members of the 'Gammaproteobacteria'. The G + C content of the genomic DNA was 63.8 mol%. The major fatty acids were branched forms, especially large proportions of iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1}ω9c, similar to the profile of the genus *Dyella*. The results of DNA–DNA hybridization with *D. japonica* XD53^T and *Frateuria aurantia* LMG 1558^T, in combination with phenotypic characteristics and 16S rRNA gene sequence analysis, demonstrated that strain BB4^T should be classified as a novel *Dyella* species. The name *Dyella koreensis* sp. nov. is proposed, with strain BB4^T (=KCTC 12359^T=NBRC 100831^T) as the type strain.

During a study of bacterial populations that metabolize ginsenoside Rb1 to Rg3, Rh2 or compound-K, 100 preliminary bacteria producing β -glucosidase were isolated from a number of sources such as human faeces, environmental soils, wastewater sludges and different types of ginseng. One of these isolates, designated strain BB4^T, represented a member of the genus *Dyella* in the family 'Xanthomonadaceae' on the basis of 16S rRNA gene sequence data; this strain was subjected to a taxonomic investigation.

The aim of this study was to determine the taxonomic position of strain BB4^T using a polyphasic approach that included physiological and DNA–DNA hybridization analyses. The results suggested that BB4^T is a representative of a novel species within the genus *Dyella*.

Strain BB4^T was isolated on aesculin agar (Atlas, 1993) at 30 °C from a soil sample around bamboo roots at the Korea Advanced Institute of Science and Technology (Daejeon, South Korea). Cell morphology and motility were observed under a Nikon light microscope (1000× magnification),

with cells grown on R2A agar for 3 days at 30 °C. Growth at different temperatures and different pH was assessed after 5 days of incubation. Salt tolerance was tested on R2A medium supplemented with 1–10% (w/v) NaCl after 5 days of incubation. Growth was determined by monitoring the OD₆₀₀. The yellow cellular pigments of our isolates were extracted with absolute ethanol from freeze-dried biomass and analysed spectrophotometrically (Gauthier & Breittmayer, 1992). The absorption spectra of crude extracts were determined in the range 200–700 nm using a spectrophotometer (DU 650; Beckman). Anaerobic growth was observed in serum bottles by adding thioglycolate (1 g l⁻¹) to R2A broth and substituting the upper air layer with nitrogen gas. Carbon-source utilization and some enzyme activities were tested by using the API 20 NE, API ID32 and API 50CHL test kits (bioMérieux). Catalase activity was determined by using 3% (v/v) H₂O₂ and oxidase activity was determined by using 1% (w/v) tetramethyl *p*-phenylenediamine. Degradation of DNA (using DNA agar from Difco, supplemented with 0.01% toluidine blue from Merck), degradation of casein, cellulose and starch (Atlas, 1993), degradation of lipid (Kouker & Jaeger, 1987) and degradation of xylan (Ten *et al.*, 2004) were also investigated; reactions were read after 5 days. Duplicate antibiotic-sensitivity tests were performed using filter-paper discs containing the following: streptomycin (5, 10

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and 15 µg ml⁻¹), tetracycline (5, 10 and 15 µg ml⁻¹), kanamycin (1.0, 1.5 and 2.0 mg ml⁻¹) and ampicillin (20, 25 and 30 µg ml⁻¹) (Sigma). Discs were placed on R2A plates spread with BB4^T culture and were then incubated at 30 °C for 5 days. Physiological and biochemical characteristics of strain BB4^T and related type strains are summarized in Table 1.

Respiratory lipoquinones were analysed as described by Komagata & Suzuki (1987). Cellular fatty acids were analysed in strain BB4^T grown on trypticase soy agar (TSA; Difco) for 48 h. The cellular fatty acids were saponified, methylated, extracted and identified by using the Microbial Identification software package (Sasser, 1990). The G + C

content of the chromosomal DNA was determined as described by Mesbah *et al.* (1989) using a reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the means of these remaining three values were used as relatedness values.

Genomic DNA was extracted and purified using the Genomic DNA isolation kit (Core Bio System Co., Ltd) and the 16S rRNA gene was PCR-amplified (Weisburg *et al.*, 1991) and directly sequenced. The 16S rRNA gene sequence

Table 1. Comparison of selected characteristics of *Dyella koreensis* sp. nov. BB4^T with those of its nearest phylogenetic neighbours within the family 'Xanthomonadaceae'

Strains: 1, *Dyella koreensis* BB4^T; 2, *Dyella japonica* XD53^T; 3, *Frateuria aurantia* LMG 1558^T (data for taxa 1–3 are from this study); 4, *Fulvimonas soli* LMG 19981^T (data from Mergaert *et al.*, 2002). +, Positive; –, negative; w, weak. All strains were positive for assimilation of glucose, growth on maltose and hydrolysis of DNA and negative for assimilation of gluconate and mannitol, growth on caprate and citrate and production of urease.

Characteristic	1	2	3	4
Motility	–	+	+	+
Oxidase	+	+	–	+
Catalase	w	+	+	+
Acid from:				
D-Glucose	+	+	+	–
D-Ribose	–	–	+	–
D-Xylose	–	–	+	–
D-Galactose	–	–	+	–
D-Mannose	–	+	+	–
L-Arabinose	–	–	+	–
Fructose	–	+	+	–
Growth on:				
N-Acetylglucosamine	+	w	–	–
D-Malate	+	–	–	–
D-Mannose	+	+	+	–
D-Sucrose	+	–	–	–
Acetate	+	–	–	+
L-Fucose	+	–	–	–
Valerate	+	–	–	–
D-Arabinose	–	–	+	–
D-Ribose	–	–	+	–
Hydrolysis of:				
Cellulose	+	–	–	+
Starch	–	–	–	+
Casein	+	–	–	+
Growth at 4% NaCl	w	–	–	+
Sensitive to kanamycin (mg ml ⁻¹)	1.0	0.9	0.7	0.6
β-Glucosidase	+	w	–	+
β-Galactosidase	+	–	–	+
Hydroxy fatty acid composition (3-OH)	iso-C _{11:0} , iso-C _{13:0} , iso-C _{17:0}	iso-C _{11:0} , iso-C _{13:0} , iso-C _{17:0}	C _{12:0}	iso-C _{11:0} , iso-C _{13:0} , iso-C _{12:0}
G + C content (mol%)	63.8	63.4–64.0	63.5	71.7

Fig. 1. Rooted phylogenetic tree based on the 16S rRNA gene sequences of strain BB4^T and related bacteria in the 'Gammaproteobacteria'. This tree was made using the neighbour-joining method (Saitou & Nei, 1987) with a two-parameter distance matrix (Kimura, 1983) and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Bar, 20 nucleotide substitutions per 1000 nucleotides.

was compiled using SEQMAN software and 16S rRNA gene sequences of the test strain and related taxa obtained from the GenBank were edited using the BIOEDIT program (Hall, 1999). Multiple alignments were performed using the CLUSTAL_X program (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed by using a neighbour-joining method (Saitou & Nei, 1987) in the program MEGA2 (Kumar *et al.*, 2001). Bootstrap analysis with 1000 replicates was used to obtain the confidence level of the branches (Felsenstein, 1985).

The nearly complete 16S rRNA gene sequence (1486 bp) of strain BB4^T was determined and subjected to comparative analysis. The 16S rRNA gene sequence of strain BB4^T showed high similarity (>97.0 %) to that of *Dyella japonica* XD53^T (Xie & Yokota, 2005) and the phylogenetic tree (Fig. 1) shows that these strains are clustered within the family 'Xanthomonadaceae' of the 'Gammaproteobacteria'. 16S rRNA gene sequence similarity values between strain BB4^T and *Frateruia aurantia* LMG 1558^T (Swings *et al.*, 1980), *Fulvimonas soli* LMG 19981^T (Mergaert *et al.*, 2002) and *Rhodanobacter lindaniclasticus* RP5557^T (Nalin *et al.*, 1999) were 97.1, 95.6 and 94.3 %, respectively. Other members of the 'Gammaproteobacteria', including members of the genera *Xanthomonas*, *Stenotrophomonas* and *Xylella*, showed less than 90 % sequence similarity.

The major respiratory lipoquinone of strain BB4^T was ubiquinone-8 (Q-8), which was also found in *D. japonica*. The fatty acid profile and G + C content of the DNA of strain BB4^T were similar to those of *D. japonica* but different from the genera *Fulvimonas* and *Frateruia*. Table 2 details the fatty acid profiles of strain BB4^T and its closest neighbours. The major fatty acids of strain BB4^T were branched forms, especially large proportions of iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1}ω9c. The G + C content of the DNA of strain BB4^T was 63.8 mol%. These phenotypic and chemotaxonomic results supported the phylogenetic analysis, which suggests that strain BB4^T belongs to the genus *Dyella*. However,

DNA–DNA hybridization experiments showed levels of relatedness of 35 and 10 % between strain BB4^T and *D.*

Table 2. Cellular fatty acid profiles of strain BB4^T and type strains of related species

Taxa: 1, *Dyella koreensis* BB4^T (data from this study); 2, *Dyella japonica* XD53^T (Xie & Yokota, 2005); 3, *Frateruia aurantia* LMG 1558^T (this study); 4, *Fulvimonas soli* LMG 19981^T (Mergaert *et al.*, 2002). 'Unknown 11.798' was an unknown peak that appeared at 11.798 min. Fatty acids that account for <0.5 % of the total are not shown; therefore, percentages do not total 100 %. –, Not detected.

Fatty acid	1	2	3	4
iso-C _{11:0}	4.3–10.4	3.8	—	4.0–5.6
Unknown 11.798	1.4–4.4	1.9	0.5–2.0	2.6–3.5
iso-C _{11:0} 3-OH	3.4–9.9	3.7	—	5.6–7.4
C _{12:0}	—	—	0.9–1.0	—
C _{12:0} 2-OH	—	—	2.3–5.1	—
C _{12:0} 3-OH	—	—	5.2–10.7	—
iso-C _{12:0} 3-OH	—	—	—	0.6–1.4
iso-C _{13:0} 3-OH	2.3–7.1	3.9	0.8–2.0	3.8–4.3
C _{14:0}	—	—	2.1–3.4	—
iso-C _{15:0}	18.2–22.6	23.6	38.2–54.7	25.8–31.2
anteiso-C _{15:0}	0.9–1.0	1.6	—	2.8–4.0
iso-C _{16:0}	2.1–2.8	4.9	—	3.7–13.2
C _{16:0}	3.2–6.4	1.5	3.8–5.6	0.4–2.2
iso-C _{17:1} ω9c	21.3–28.1	25.6	2.3–2.5	15.8–21.7
iso-C _{17:0}	15.6–26.0	20	1.8–4.4	10.9–15.0
anteiso-C _{17:0}	—	0.7	—	0.6–1.1
iso-C _{17:0} 3-OH	0.8–1.1	1.1	—	—
C _{17:0} cyclo	—	—	2.2–6.3	—
C _{17:1} ω6c	—	—	—	0.3–1.5
C _{18:0}	1.1–1.5	—	—	—
iso-C _{19:0}	1.6–1.8	0.7	—	—
Summed feature 3*	4.9–7.0	4.0	9.3–13.0	2.0–2.8

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

japonica XD53^T and *Frateuria aurantia* LMG 1558^T, respectively, suggesting that strain BB4^T should be classified as representing a novel species (Wayne *et al.*, 1987).

Cells of strain BB4^T were aerobic, Gram-negative, non-motile, straight rods, 0.2–0.5 µm in diameter and 1.5–2.1 µm in length. Colonies formed on R2A medium (Difco) were yellow, convex, transparent with clear edges. The absorption spectra of pigments from the strain showed a maximum peak at 425 nm. The strain was oxidase-positive and weakly catalase-positive. It did not grow anaerobically but could reduce nitrate to nitrite. Strain BB4^T grew at 10–37 °C, with an optimum at 30 °C. The optimum pH for growth was in the range 7.0–8.0. As shown in Table 1, BB4^T showed positive results for β-glucosidase and β-galactosidase. It showed growth in R2A medium containing 4% (w/v) NaCl. Details of various differentiating characteristics of strain BB4^T and phylogenetically related species are shown in Table 1; other characteristics determined are given in the species description.

On the basis of phylogenetic and physiological data, we propose that isolate BB4^T should be classified as the type strain of a novel species, *Dyella koreensis* sp. nov.

Description of *Dyella koreensis* sp. nov.

Dyella koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* pertaining to Korea, the location of the soil sample from which the type strain was isolated).

Gram-negative, aerobic, non-motile, rod-shaped cells. Colonies on R2A agar plates are smooth, circular, transparent and yellow in colour within 5 days at 30 °C. Optimal growth occurs at 30 °C and pH 6.8–7.5. It does not grow anaerobically. Growth occurs with several carbon sources, such as acetate, D-fucose, D-glucose, 3-hydroxybutyrate, malate, maltose, mannose, D-melibiose, N-acetylglucosamine, salicin, D-sucrose and valerate. However, it shows negative results for adipate, L-alanine, L-arabinose, caprate, citrate, glycogen, gluconate, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, itaconate, inositol, lactate, malonate, mannitol, phenylacetate, L-proline, propionate, rhamnose, D-ribose, L-serine, D-sorbitol and suberate. It can hydrolyse aesculin, cellulose, casein and DNA, produces β-galactosidase and β-glucosidase and can reduce nitrate. The G + C content of the genomic DNA is 63.8 mol%. Ubiquinone-8 is the main respiratory lipoquinone. Major fatty acids are iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1}ω9c when grown on TSA for 48 h.

The type strain is BB4^T (= KCTC 12359^T = NBRC 100831^T).

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