

Arthrobacter soli sp. nov., a Novel Bacterium Isolated from Wastewater Reservoir Sediment

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A novel Gram-positive bacterium, designated SYB2^T, was isolated from wastewater reservoir sediment, and a polyphasic taxonomic study was conducted based on its morphological, physiological, and biochemical features, as well as the analysis of its 16S rRNA gene sequence. During the phylogenetic analysis of the strain SYB2^T, results of a 16S rRNA gene sequence analysis placed this bacterium in the genus *Arthrobacter* within the family *Micrococcaceae*. SYB2^T and *Arthrobacter protophormiae* ATCC 19271^T, the most closely related species, both exhibited a 16S rRNA gene sequence similarity of 98.99%. The genomic DNA G+C content of the novel strain was found to be 62.0 mol%. The predominant fatty acid composition was anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0}, and iso-C_{15:0}. Analysis of 16S rRNA gene sequences and DNA-DNA relatedness, as well as physiological and biochemical tests, showed genotypic and phenotypic differences between strain SYB2^T and other *Arthrobacter* species. The type strain of the novel species was identified as SYB2^T (= KCTC 19291^T= DSM 19449^T).

Keywords: *Arthrobacter soli* sp. nov., taxonomy, wastewater reservoir sediment

Bacterium globiformis was first reported by Conn (1928), and was later classified as *Arthrobacter globiforme* within the phylum *Actinobacteria* by Conn and Dimmick (1947). The genus *Arthrobacter*, which belongs to the family *Micrococcaceae*, currently contains more than 51 species (<http://www.bacterio.cict.fr/a/arthrobacter.html>) and was isolated from diverse environments, including regions within Antarctica (Reddy *et al.*, 2002; Gupta *et al.*, 2004; Chen *et al.*, 2005), certain kinds of cheese (Irlinger *et al.*, 2005), various clinical specimens (Funke *et al.*, 1996; Hou *et al.*, 1998; Wauters *et al.*, 2000; Huang *et al.*, 2005), pond water (Reddy *et al.*, 2000), soil (Lee *et al.*, 2003), paintings (Heyrman *et al.*, 2005), seals (Collins *et al.*, 2002), an alpine ice cave (Margesin *et al.*, 2004), fish (Osorio *et al.*, 1999), and air (Li *et al.*, 2004). This paper aims to establish the taxonomic position of strain SYB2^T (T=type strain), which was isolated from wastewater reservoir sediment, using phenotypic, genetic, and chemotaxonomic analyses.

Materials and Methods

Bacterial strains

The wastewater reservoir sediment, from which Strain SYB2^T was isolated, was collected in Daejeon (Republic of Korea). The isolate was grown on TSBA medium [tryptic soy broth/L

solidified with 20.0 g agar/L (Difco, USA)] at 30°C, and repeatedly re-streaked to obtain a pure culture. Strain SYB2^T was deposited into the KCTC (Korean Collection for Type Cultures) as KCTC 19291^T as well as the DSMZ (German Collection of Microorganisms and Cell Cultures) as DSM 19449^T. Reference strains used for DNA-DNA hybridization tests included *Arthrobacter ardeleyensis* JCM 12921^T, *A. ari-laitensis* JCM 13566^T, and *A. bergerei* JCM 13567^T, obtained from JCM (Japan Collection of Microorganisms), and *A. creatinolyticus* KCTC 9903^T, *A. mysorens* KCTC 3381^T, *A. nicotianae* KCTC 3382^T, *A. protophormiae* KCTC 3385^T, and *A. uratoxydans* KCTC 3482^T, obtained from KCTC.

Morphology and physiological characteristics

Cell morphology was examined by light microscopy (ECLIPSE 80i, Nikon, Japan), and Gram-staining was performed using the non-staining method described by Buck (1982). Cells were grown on TSBA to determine growth conditions for various temperatures (4, 10, 15, 16, 17, 20, 25, 30, 37, 40, 41, 42, 45, 50, and 60°C) and in TSB for pH (5.0~12.0 at intervals of 0.5 pH units) analysis, adjusted with HCl or NaOH. Tolerance to NaCl% (w/v) was determined using marine broth (MB, Difco) media supplemented with appropriate concentrations of NaCl. Acid production from carbohydrate, utilization of sole carbon sources, and enzyme activities were determined using API20NE, API50CH, and API ZYM test strips (BioMerieux Inc., France). Oxidase activity was determined using an oxidase reagent (BioMerieux Inc.), and bubble production in a 3% (v/v) hydrogen peroxide

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solution was used to assess catalase activity of this strain.

Chemotaxonomy

The cell biomass for cellular fatty acid composition analysis was collected from TSBA plates after incubation for 2 days. Cells were harvested, and the cellular fatty acids were saponified, methylated and extracted, following the instructions in the manual for the Sherlock Microbial Identification System (MIDI, USA). The fatty acids were analyzed by gas chromatography (Hewlett Packard 6890, USA) and identified using the Microbial Identification software package (Sasser, 1990).

Determination of G+C content, 16S rRNA gene sequencing, and phylogenetic analysis

Chromosomal DNA was extracted using a G-spin™ DNA extraction kit (iNtRON Biotechnology, Korea), and the G+C content was determined using high-performance liquid chromatography (HPLC). *E. coli* B (Sigma-Aldrich, USA) was used as the calibration reference, as described by Mesbah and Whitman (1989). The 16S rRNA gene was PCR-amplified from chromosomal DNA using a PCR Pre-Mix (Solgent, Korea). Two universal primers were used for amplification: a forward primer 27f; 5'-AGAGTTTGTATCCTGGCTCAG-3', which was specific for most bacteria, and a reverse primer 1492r; 5'-TACGGYTACCTTGTTACGACTT-3', which was specific for most bacteria and archaea (Lane, 1991). PCR was initiated by denaturation at 94°C for 2 min. Reactions were amplified for 30 cycles as follows; denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1.5 min. A final extension was performed at 72°C for 10 min. The PCR products were purified and sequenced using a PCR purification kit (Cosmo genetech, Korea) and the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA), respectively, according to the manufacturer's instructions. An automated DNA analyzer

system (PRISM 3730XL DNA analyzer, Applied Biosystems) was used to analyze the resulting reaction mixtures, and the full-length 16S rRNA gene sequences were assembled using SeqMan software (DNASTar, USA).

Pair-wise 16S rRNA gene sequence similarity was determined using the EzTaxon server [http://www.eztaxon.org/; Chun *et al.* (2007)] to locate phylogenetic neighbors. Sequences from the novel strain and related taxa (NCBI database) were aligned using the multiple sequence alignment program CLUSTAL X ver. 1.8 (Thompson *et al.*, 1997). Phylogenetic relationships between representative *Arthrobacter* species were determined using the MEGA3 software program (Kumar *et al.*, 2004). Distance matrices were determined (Kimura, 1980), and used to elaborate dendrograms by the neighbor-joining (Saitou and Nei, 1987) method. To evaluate the stability of the phylogenetic tree that was generated, a bootstrap analysis was performed using a consensus tree based on 1,000 randomly generated trees (Felsenstein, 1985).

Nucleotide sequence accession number

The SYB2^T 16S rRNA gene sequence is available from the GenBank nucleotide database at the NCBI website (http://www.ncbi.nlm.nih.gov) under accession number EF660748.

DNA-DNA hybridization

A DNA-DNA homology experiment was performed using the modified method of Ezaki *et al.* (1989), using a non-treated, polystyrene, black microplate (MaxiSorp, FluoroNunc, Denmark) and measured fluorometrically. DNA and buffer mixtures were dispensed into the wells of a microplate such that each well contained 1 µg of DNA. All reactions were performed in quintuplicate experiments. Of the five separate experiments, the highest and lowest value of fluorescence intensity was excluded, and the three remaining values were averaged to generate the DNA-DNA homology value. The fluorescence intensity value obtained for the homolo-

Table 1. Differential characteristics of *Arthrobacter soli* and closely related species

Characteristic	1	2	3	4	5	6
Growth at 15°C	-	+	+	+	+	+
Growth at 37°C	+	+	+	-	-	-
Maximum temperature (°C)	40	37	37	30	30	30
Growth at 10% NaCl	+	-	-	+	-	+
Nitrate to nitrite reduction	+	+	+	-	-	+
Gelatinase	+	+	d	+	-	NR
β-Galactosidase	-	-	-	+	+	NR
Utilization of:						
D-Xylose	-	-	+	+	-	+
D-Ribose	+	-	+	+	+	+
L-Arabinose	-	-	+	+	+	+
D-Galactose	+	-	-	+	-	W
L-Rhamnose	-	-	-	-	-	+

Strains: 1, *Arthrobacter soli* SYB2^T sp. nov.; 2, *A. protophormiae* ATCC 19271^T (Data from Stackebrandt *et al.*, 1983); 3, *A. nicotianae* AS 1.1895^T (Stackebrandt *et al.*, 1983); 4, *A. arilaitensis* Re117^T (Irlinger *et al.*, 2005); 5, *A. bergerei* Ca106^T (Irlinger *et al.*, 2005); 6, *A. ardleyensis* An25^T (Chen *et al.*, 2005). +, positive; -, negative; d, reaction differs among strains; NR, not reported; W, weak positive.

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

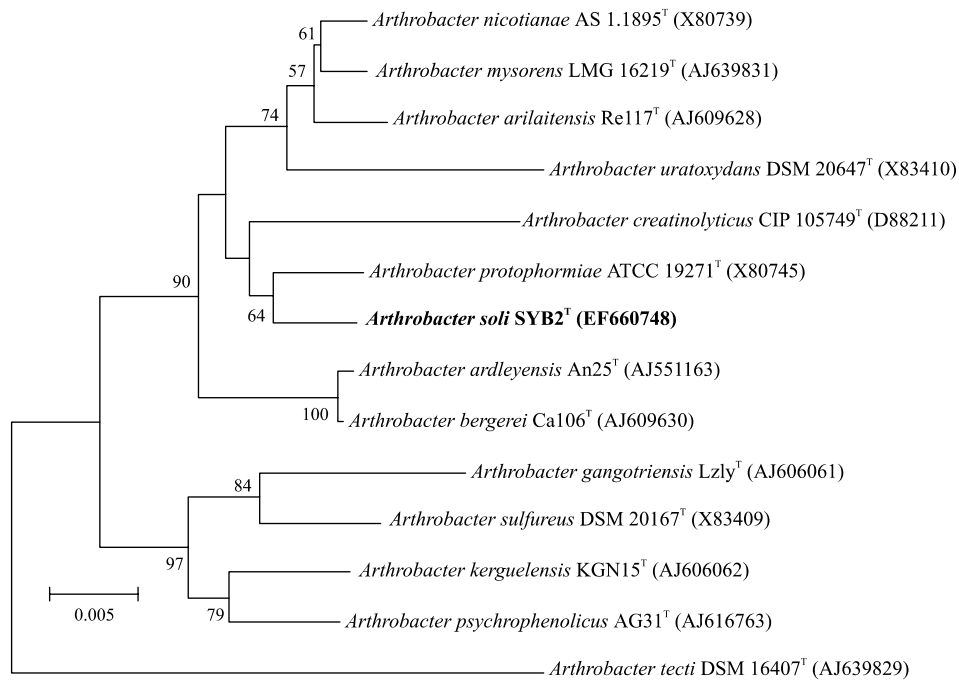


Fig. 1. Neighbor-joining phylogenetic tree showing positions of *Arthrobacter soli* SYB2^T and its related species. The relationships are based on 16S rRNA gene sequence analysis. Numbers at the nodes indicate bootstrap values (expressed as percentages of 1,000 replications). Bootstrap values greater than 50% are shown at the branch points. Bar, 0.005 accumulated changes per nucleotide.

gous reaction well was designated as the 100% homology relationship.

Results and Discussion

Morphology and physiological characteristics

Strain SYB2^T is a Gram-positive bacterium, exhibiting yellow-pigmented colonies (2 to 3 mm in diameter) on TSBA. It can grow in NaCl concentrations up to 10% (w/v) and in temperatures up to 40°C. The strain was found to be oxidase-positive and catalase-negative, and was able to reduce nitrate to nitrite. It was unable to produce indole or ferment glucose. Strain SYB2^T did not react with arginine dihydrolyase and urease, and was able to hydrolyse gelatin but not aesculin and PNPG (*p*-Nitrophenyl α -D-glucopyranoside). Additional description of the strain is given in the species description, and Table 1 shows a comparison between the characteristics of SYB2^T and other closely related strains.

Chemotaxonomy

The predominant fatty acid composition of the strain SYB2^T includes anteiso-C_{15:0} (53.73%), anteiso-C_{17:0} (15.81%), iso-C_{16:0} (12.32%), iso-C_{15:0} (9.74%), C_{16:0} (3.23%), iso-C_{17:0} (2.27%), iso-C_{14:0} (1.66%), with additional fatty acids comprising less than 1%. This composition profile with anteiso-C_{15:0} as a predominant fatty acid is typical of members of the genus *Arthrobacter* (Lee *et al.*, 2003; Storms *et al.*, 2003; Kotouckova *et al.*, 2004; Margesin *et al.*, 2004; Heyrman *et al.*, 2005; Tvrzova *et al.*, 2005). This therefore confirms that SYB2^T belongs to the genus *Arthrobacter*.

Phylogenetic analysis

The 16S rRNA gene sequence similarity, determined using the EzTaxon server, demonstrated the association of the novel strain SYB2^T with the genus *Arthrobacter*. Type strains closely related phylogenetically to strain SYB2^T were found to be *Arthrobacter protophormiae* ATCC 19271^T (98.99%), *A. nicotianae* AS 1.1895^T (98.49%), *A. mysorens* LMG 16219^T (98.42%), *A. arilaitensis* Re117^T (98.28%), *A. bergerei* Ca106^T (98.27%), *A. ardleyensis* An25^T (98.20%), *A. psychrophenicus* AG31^T (97.27%), *A. uratoxydans* DSM 20647^T (97.12%), and other type strains in the genus *Arthrobacter* ($\leq 96.83\%$).

A phylogenetic tree (Fig. 1) constructed using the neighbor-joining method from the 16S rRNA gene sequence analysis indicated that the strain SYB2^T belonged within a cluster of *Arthrobacter*, sharing high 16S rRNA gene sequence similarity.

G+C content and DNA-DNA hybridization

The genomic DNA G+C content of strain SYB2^T was calculated to be 62.0 mol%, while the reported G+C content of *Arthrobacter woluwensis* CUL 1808^T and *A. ardleyensis* An25^T were calculated to be 55.2–59.5 mol% (Chen *et al.*, 2005) and 69 mol% (Funke *et al.*, 1996), respectively. Thus, the genomic DNA G+C content of strain SYB2^T was found to be within the range of those validated in *Arthrobacter* species.

DNA-DNA hybridization tests were performed to determine the genomic relatedness between strain SYB2^T and 8 of its closest relatives, clustered within the phylogenetic tree and possessing a high 16S rRNA gene sequence similarity $\geq 97.0\%$. The results showed an average level of

DNA-DNA relatedness below the 70% threshold value (Wayne *et al.*, 1987) for strain SYB2^T and each of its closest relatives: *A. protophormiae* (19.1%), *A. nicotianae* (18.8%), *A. mysorens* (29.7%), *A. arilaitensis* (25.1%), *A. bergerei* (22.7%), *A. ardleyensis* (20.9%), *A. uratoxydans* (31.3%), and *A. creatinolyticus* (27.4%). The characteristics of the 16S rRNA gene sequence similarity and low DNA-DNA relatedness values indicate that strain SYB2^T represents a distinct genospecies.

Therefore, based on polyphasic comparisons to previously described taxa, strain SYB2^T was deduced to be a type strain of a novel species belonging to the genus *Arthrobacter*, for which the name *Arthrobacter soli* sp. nov. is proposed.

Description of *Arthrobacter soli* sp. nov.

Arthrobacter soli (so'li. L. neut. gen. n. soli of soil, the source of the type strain)

The cells are rod-shaped (0.9 by 2.0~3.0 µm), Gram-positive, oxidase-positive, and catalase-negative. Colonies are yellow-pigmented and circular, measuring approximately 1.0~2.0 mm in diameter after 2 days of growth on TSBA at 30°C. The temperature range for growth is 16~40°C, but no growth occurs at 15 and 41°C. No growth occurs in NaCl concentrations greater than 15%. Optimal pH for growth is 7.0. The strain can reduce nitrate to nitrite. Indole is not produced, and glucose fermentation does not occur. Cells are arginine dihydrolase- and urease-negative. Gelatin hydrolysis occurs, but aesculin and PNPG (*p*-Nitrophenyl α -D-glucopyranoside) hydrolysis do not occur. Potassium gluconate, malate, trisodium citrate, phenylacetic acid, glycerol, L-arabinose, D-ribose, D-adonitol, methyl- β -D-xyloside, D-galactose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, D-cellobiose, D-maltose, D-lactose, gluconate, 2-ketogluconate and 5-ketogluconate are assimilated; capric acid, adipic acid, erythritol, D-arabinose, D-xylose, L-xylose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl- α -D-mannoside, methyl- α -D-glucoside, amygdalin, arbutin, esculin, salicin, D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol and L-arabitol are not assimilated. Cells are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and α -mannosidase. However cells are negative for α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, and α -fucosidase.

The predominant fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}, iso-C_{16:0}, and iso-C_{15:0}. The G+C content of genomic DNA of the type strain is 62.0 mol%. The type strain is SYB2^T (= KCTC 19291^T = DSM 19449^T), which was isolated from wastewater reservoir sediment collected in Daejeon, Republic of Korea.

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