# *Georgenia daeguensis* sp. nov., isolated from 4-chlorophenol enrichment culture

Sung-Geun Woo,[1,2,3] Yingshun Cui,[4] Myung-Suk Kang,[5] Long Jin,[6] Kwang Kyu Kim,[7] Sung-Taik Lee,[4] Myungjin Lee[3] and Joonhong Park[1,2]

Correspondence
Joonhong Park
parkj@yonsei.ac.kr

[1]School of Civil and Environmental Engineering, Yonsei University, Seoul 120-749, Republic of Korea

[2]WCU Center for Green Metagenomics, Yonsei University, Seoul 120-749, Republic of Korea

[3]Research and Development Division, H-Plus Eco Ltd, BVC 301, KRIBB, Eoeun-dong, Yuseong-gu, Daejeon 305-333, Republic of Korea

[4]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea

[5]National Institute of Biological Resources, Environmental Research Complex, Gyeongseo-dong, Seo-gu, Incheon 404-708, Republic of Korea

[6]Environmental Biotechnology Research Center, Korea Research Institute of Bioscience & Biotechnology, 52 Eoeun-dong, Yuseong-gu, Daejeon 305-806, Republic of Korea

[7]Korean Collection for Type Cultures, Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, 52 Eoeun-dong, Yuseong-gu, Daejeon 305-806, Republic of Korea

During screening for 4-chlorophenol-degrading micro-organisms in activated sludge from industrial wastewater treatment, a Gram-positive, rod-shaped, aerobic bacterial strain, designated 2C6-43$^T$, was isolated and characterized taxonomically by using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that strain 2C6-43$^T$ belongs to the family *Bogoriellaceae*, class *Actinobacteria*, and is related most closely to *Georgenia soli* CC-NMPT-T3$^T$ (98.8 % sequence similarity), *Georgenia muralis* 1A-C$^T$ (97.6 %), *Georgenia thermotolerans* TT02-04$^T$ (96.8 %), *Georgenia ruanii* YIM 004$^T$ (96.6 %) and *Georgenia halophila* YIM 93316$^T$ (96.0 %). The G+C content of the genomic DNA of strain 2C6-43$^T$ was 66.2 mol%. Sugars from whole-cell hydrolysates found in strain 2C6-43$^T$ were rhamnose, ribose and galactose. The menaquinone MK-8($H_4$) was detected as the predominant quinone. Polar lipid analysis of 2C6-43$^T$ revealed diphosphatidylglycerol, phosphatidylinositol mannoside, phosphatidylinositol and phosphatidylglycerol. An aromatic compound ring cleavage enzyme of catechol 1,2-dioxygenase was detected but catechol 2,3-dioxygenase was not detected in 2C6-43$^T$. A fatty acid profile with anteiso-$C_{15:0}$, iso-$C_{15:0}$ and $C_{16:0}$ as the major components supported the affiliation of strain 2C6-43$^T$ to the genus *Georgenia*. However, the DNA–DNA relatedness between strain 2C6-43$^T$ and the type strains of five species of the genus *Georgenia* ranged from 17 to 40 %, clearly showing that the isolate constitutes a new genospecies. Strain 2C6-43$^T$ could be clearly differentiated from its phylogenetic neighbours on the basis of some phenotypic, genotypic and chemotaxonomic features. Therefore, strain 2C6-43$^T$ is considered to represent a novel species of the genus *Georgenia*, for which the name *Georgenia daeguensis* sp. nov. is proposed; the type strain is 2C6-43$^T$ (=KCTC 19801$^T$=JCM 17459$^T$).

Abbreviation: CP, chlorophenol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 2C6-43$^T$ is HQ246163.

Two supplementary figures are available with the online version of this paper.

The genus *Georgenia*, a member of the family *Bogoriellaceae*, class *Actinobacteria*, was proposed in 2002 by Altenburger *et al.* (2002). At the time of writing, the genus *Georgenia* comprised five species with validly published names: *Georgenia muralis* (Altenburger *et al.*, 2002), *G. ruanii* (Li *et al.*, 2007), *G. thermotolerans* (Hamada *et al.*,

2009), *G. soli* (Kämpfer *et al.*, 2010) and *G. halophila* (Tang *et al.*, 2010). Members of the genus *Georgenia* have the following characteristics: they are Gram-positive, non-endospore-forming, motile or non-motile rods, positive for catalase and oxidase, contain anteiso-$C_{15:0}$ as the predominant cellular fatty acid and DNA G+C contents range from 69.7 to 72.9 mol%. During screening for 4-chlorophenol (4-CP)-degrading micro-organisms from activated sludge from industrial wastewater treatment, a Gram-positive, rod-shaped bacterial strain, designated 2C6-43$^T$, was isolated. On the basis of 16S rRNA gene sequence analysis, this isolate was considered to be a *Georgenia*-like strain. To determine its exact taxonomic position, 2C6-43$^T$ was subjected to a detailed investigation using a polyphasic taxonomic approach, including genotypic, chemotaxonomic and classical phenotypic analyses (Tindall *et al.*, 2010). These results indicate that 2C6-43$^T$ should be placed in the genus *Georgenia* as a representative of a novel species.

An activated sludge sample was initially incubated with 50 p.p.m. (0.39 mM) 4-CP and diluted serially in 0.85 % saline solution. Aliquots of each serial dilution were spread on R2A agar and incubated at 30 °C for 14 days. Single colonies on the plates were purified by transferring them onto fresh plates and incubating them again under the same conditions. The isolate was routinely cultured at 30 °C on R2A agar [containing (per l distilled water) 0.5 g glucose, 0.5 g soluble starch, 0.5 g casein hydrolysate, 0.5 g yeast extract, 0.5 g peptone, 0.05 g $MgSO_4.7H_2O$, 0.3 g $KH_2PO_4$ and 15 g agar, with the pH adjusted to 7.2 prior to autoclaving] and maintained as a glycerol suspension (20 %, w/v) at −70 °C. *G. halophila* DSM 21365$^T$, *G. muralis* DSM 14418$^T$, *G. ruanii* KCTC 19029$^T$, *Georgenia soli* DSM 21838$^T$ and *Georgenia thermotolerans* DSM 21501$^T$ were used as reference strains for DNA–DNA hybridization and other experiments.

The Gram reaction was determined by using a Gram-stain kit (Difco) according to the manufacturer's instructions. Cell morphology and motility were observed under an Olympus light microscope (×1000 magnification) using the hanging drop technique with cells grown for 2 days at 30 °C on R2A agar. The presence of endospores was examined by staining using the method of Cappuccino & Sherman (2002) and cells were observed using light microscopy. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). Assimilation of single carbon sources, enzyme activities and other physiological characteristics were determined with the API ID 32 GN (48 h, 30 °C) and API 20 NE (48 h, 30 °C) galleries according to the manufacturer's instructions (bioMérieux) with cells grown on Luria–Bertani (LB) medium for 2 days. Reduction of nitrate and nitrite was determined using serum bottles (25 ml) containing 12 ml R2A broth supplemented with $KNO_3$ (10 mM) and $NaNO_2$ (10 mM), respectively (Assih *et al.*, 2002). The reduction of nitrate and nitrite was monitored by ion chromatography on a model 790 personal IC (Metrohm) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). Growth at different temperatures (4, 10, 15, 20, 25, 30, 37 and 42 °C) was assessed on R2A agar, nutrient agar (NA), LB agar, trypticase soy agar (TSA; Difco) and MacConkey agar after 5 days incubation. Salt tolerance was tested on R2A medium supplemented with 0–15 % (w/v) NaCl after incubation for up to 5 days. Growth at various pH values (pH 5.0–11.0 at intervals of 1.0 pH unit) was evaluated in R2A broth at 30 °C.

For phylogenetic analysis of strain 2C6-43$^T$, genomic DNA was extracted by using a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). The almost complete sequences of 16S rRNA genes used were compiled by using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by using the program CLUSTAL_X (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated by using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms from MEGA4 (Tamura *et al.*, 2007) and the maximum-likelihood (Felsenstein, 1981) method from PHYLIP version 3.69 (Felsenstein, 2009), with bootstrap values based on 1000 replications (Felsenstein, 1985).

For the determination of chromosomal DNA G+C content, the genomic DNA of the strain was extracted and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides; the DNA G+C content was determined as described by Mesbah *et al.* (1989) using reverse-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane. The crude quinone in n-hexane was purified using Sep-Pak Vac silica cartridges (Waters) and then analysed by HPLC, as described previously (Hiraishi *et al.*, 1996). Cellular fatty acids were analysed using cells grown on TSA for 48 h at 30 °C. The cellular fatty acids were liberated, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI) version 3.0 (Sasser, 1990). The fatty acid methyl esters were then analysed by GC (Agilent 6890; Hewlett Packard) using the Aerobe (TSBA6, version 6.0) database of the Sherlock Microbial Identification software package. Polar lipids were extracted using the procedure described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents as described previously (Lee *et al.*, 2008). Purified cell-walls were obtained by disruption of cells with glass beads and subsequent trypsin digestion, according to the method of Schleifer (1985). Amino acids and peptides in the cell-wall hydrolysates were analysed by using two-dimensional TLC on cellulose plates with the solvent

systems described by Schleifer & Kandler (1972) and the HPLC system described by Tang *et al.* (2009). Sugars from whole-cell hydrolysates were analysed as described by Staneck & Roberts (1974). The utilization of monochlorophenols as sole carbon sources was determined by using a minimum salt medium (Bae *et al.*, 1996) with 2 % Noble agar (Difco). After sterilization, 2-CP (100, 200, 300, 400 and 500 p.p.m.), 3-CP (100, 200, 250, 300 and 350 p.p.m.) and 4-CP (100, 150, 200, 250 and 300 p.p.m.) were added separately. On these media, 2C6-43$^T$ was incubated for 21 days. To minimize the evaporation of phenolic compounds, this test was performed in 160 ml serum bottles with butyl rubber stoppers. After sterilization, aromatic compounds phenol 2-CP (10, 25, 50 p.p.m.), 3-CP (10, 25, 50 p.p.m.) and 4-CP (10, 25, 50 p.p.m.) were added separately. The change of aromatic compounds in liquid media was detected as described previously (Bae *et al.*, 1996). A wide variety of catechol 1,2-dioxygenase and catechol 2,3-dioxygenase genes were detected as described previously (Sei *et al.*, 1999).

Cells of strain 2C6-43$^T$ were Gram-positive, aerobic and motile rods. Colonies grown on LB agar plates for 2 days at 30 °C were 1–2 mm in diameter, circular, convex, shiny, opaque and yellowish. On LB agar, strain 2C6-43$^T$ was able to grow at 10–42 °C, but not at 4 °C. Normal growth was also observed on R2A, TSA and NA at 20–37 °C and weak growth occurred on these media at 10 and 42 °C. On MacConkey agar, no growth was observed at any temperature. As a result of salt tolerance tests and pH range test, strain 2C6-43$^T$ was grown on R2A agar media with 9 % (w/v) NaCl and in R2A broth with pH range of 5–10. Strain 2C6-43$^T$ and strains of five *Georgenia* species (*G. halophila* DSM 21365$^T$, *G. muralis* DSM 14418$^T$, *G. ruanii* KCTC 19029$^T$, *G. soli* DSM 21838$^T$ and *G. thermotolerans* DSM 21501$^T$) used in this study as reference strains, exhibited positive enzymic activities for catalase, oxidase, $\beta$-glucosidase and $\beta$-galactosidase; they were also positive for assimilation of glucose, sucrose, maltose, gluconate and glycogen. All the above-mentioned strains were negative for: arginine dihydrolase, urease and indole production; assimilation of L-histidine, L-serine, L-proline, D-sorbitol, inositol, caprate, adipate, citrate, phenylacetate, sodium malonate, lactate, propionate, valerate, trisodium citrate and 4-hydroxybenzoate; and acidification of glucose. These results are in good agreement with previously reported data and supported the affiliation of the isolate to the genus *Georgenia* (Altenburger *et al.*, 2002; Li *et al.*, 2007; Hamada *et al.*, 2009; Kämpfer *et al.*, 2010; Tang *et al.*, 2010). Phenotypic and chemotaxonomic characteristics that differentiate strain 2C6-43$^T$ from its closest neighbours in the genus *Georgenia* are listed in Table 1. In particular, strain 2C6-43$^T$ could be clearly differentiated from all above-mentioned *Georgenia* species based on its ability to utilize salicin and D,L-3-hydroxybutyrate. Utilization of monochlorophenols by strain 2C6-43$^T$ as sole carbon sources in solid phase of minimum salt medium is shown in Table 2. However, in an aqueous phase of the same basal medium, no utilization of aromatic compounds was observed and there was no growth of bacteria over 21 days of incubation. As a result of analysis of two aromatic compound ring cleavage enzymes (catechol 1,2-dioxygenase and catechol 2,3-dioxygenase) which are involved in the oxidation of CPs, catechol 1,2-dioxygenase was detected in 2C6-43$^T$ but catechol 2,3-dioxygenase was not detected.

The 16S rRNA gene sequence of strain 2C6-43$^T$ was a continuous stretch of 1382 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain 2C6-43$^T$ were *G. soli* DSM 21838$^T$ (98.8 % sequence similarity), *G. muralis* DSM 14418$^T$ (97.6 %), *G. thermotolerans* DSM 21501$^T$ (96.8 %), *G. ruanii* KCTC 19029$^T$ (96.6 %) and *G. halophila* DSM 21365$^T$ (96.0 %). The phylogenetic position of the isolate, determined by using the various tree-making algorithms (neighbour-joining, maximum-parsimony and maximum-likelihood), revealed that strain 2C6-43$^T$ appeared within the genus *Georgenia* with a high bootstrap value of 96 % (Fig. 1). The generally accepted criteria for delineating bacterial species state that strains with DNA–DNA relatedness below 70 % (as measured from hybridization) or with a 16S rRNA gene sequence dissimilarity above 3 % are considered to belong to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002). In view of this definition, the above-mentioned data indicate that strain 2C6-43$^T$ can be considered to represent a novel species of the genus *Georgenia*. For further verification of the taxonomic position of strain 2C6-43$^T$, DNA–DNA hybridization was performed with the most closely related members of the genus *Georgenia*.

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes (Sigma) and microdilution wells (Greiner), with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as the DNA–DNA hybridization values. Strain 2C6-43$^T$ exhibited relatively low levels of DNA–DNA relatedness with respect to *G. soli* DSM 21838$^T$ (40.5 %), *G. muralis* DSM 14418$^T$ (29.7 %), *G. halophila* DSM 21365$^T$ (20.5 %), *G. thermotolerans* DSM 21501$^T$ (19.2 %) and *G. ruanii* KCTC 19029$^T$ (17.6 %) indicating that it is not related to them at the species level (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

Cellular fatty acid profiles of strain 2C6-43$^T$ and the five reference strains are shown in Table 3. All strains contained anteiso-$C_{15:0}$ as the dominant component and also contained $C_{16:0}$, $C_{18:0}$ and iso-$C_{15:0}$ as the major components. However, some qualitative and quantitative differences in fatty acid content were observed between strain 2C6-43$^T$ and the reference strains. Strain 2C6-43$^T$ contained a comparatively high amount of anteiso-$C_{15:1}$ A, whereas other species have smaller amounts or none of this fatty acid. In particular, strain 2C6-43$^T$ contained a small amount of the hydroxylated fatty acid $C_{12:0}$ 3-OH, which

**Table 1.** Differential phenotypic characteristics of strain 2C6-43$^T$ and its closest phylogenetic neighbours in the genus *Georgenia*

Strains: 1, 2C6-43$^T$; 2, *G. soli* DSM 21838$^T$; 3, *G. muralis* DSM 14418$^T$; 4, *G. thermotolerans* DSM 21501$^T$; 5, *G. ruanii* KCTC 19029$^T$; 6, *G. halophila* DSM 21365$^T$. All data are from the present study. All strains are positive for aesculin hydrolysis and *p*-nitrophenyl-β-D-galactopyranoside but negative for arginine dihydrolase, urease, indole production and glucose acidification. In substrate assimilation tests, all strains showed identical biochemical characteristics except those indicated here. +, Positive; w, weakly positive; −, negative.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Motility | + | + | − | + | + | − |
| Morphology | Short rod | Coccoid | Coccoid rod | Rod | Short rod | Short rod |
| Size (μm) | | | | | | |
| Length | 0.5–0.8 | 0.8–1.2 | 0.8–2.0 | 1.0 | 1.5–2.5 | 0.3–0.8 |
| Width | 0.3–0.5 | 0.8–1.2 | 0.8 | 0.5–0.8 | 0.5–0.8 | 0.2–0.5 |
| Colony colour | Yellow | Pale yellow | Yellow | Pale yellow | Pale yellow | Yellow |
| Growth temperature (°C) | | | | | | |
| Range | 10–42 | 15–37 | 10–42 | 10–42 | 10–37 | 25–42 |
| Optimum | 25–30 | 25–30 | 30–37 | 25–30 | 30 | 37 |
| NaCl range (%) | 1–9 | 1–9 | 1–8 | 1–7 | 1–4 | 1–15 |
| Growth pH | | | | | | |
| Range | 5–10 | 5–8 | 5–9 | 5–9 | 6–9 | 5–8 |
| Optimum | 6–8 | 7–8 | 7–8 | 6–8 | 7–8 | 6–8 |
| Reduction of: | | | | | | |
| Nitrate | + | + | + | + | − | + |
| Nitrite | − | − | − | − | − | − |
| Gelatin hydrolysis | − | + | − | − | − | − |
| Assimilation of: | | | | | | |
| 3-Hydroxybenzoate | − | − | − | − | − | + |
| Potassium 2-ketogluconate | − | + | + | − | − | − |
| Potassium 5-ketogluconate | + | + | + | − | − | − |
| D,L-3-Hydroxybutyrate | + | − | − | − | − | − |
| L-Rhamnose | − | − | − | − | + | − |
| L-Arabinose | − | − | + | − | + | − |
| L-Fucose | − | + | − | − | w | − |
| D-Ribose | + | + | − | + | − | + |
| D-Mannose | + | − | − | + | − | + |
| Melibiose | − | − | + | − | + | − |
| D-Mannitol | − | − | + | − | + | − |
| L-Alanine | − | − | − | − | − | + |
| Salicin | − | + | + | + | + | + |
| Malate | − | − | − | − | − | + |
| Itaconate | − | − | − | − | − | + |
| Suberate | − | − | − | − | − | + |
| Sodium acetate | − | − | + | w | − | w |
| *N*-Acetylglucosamine | + | + | + | − | + | + |

was not present in any of the other strains. Strain 2C6-43$^T$ contained MK-8($H_4$) as the predominant menaquinone. The DNA G+C content of strain 2C6-43$^T$ was 66.2 mol%. These data are in good agreement with those of other members of the genus *Georgenia* (Altenburger *et al.*, 2002; Li *et al.*, 2007; Hamada *et al.*, 2009; Kämpfer *et al.*, 2010; Tang *et al.*, 2010). The amino acids glutamic acid, alanine and lysine were detected in total hydrolysates of the cell-wall preparation of strain 2C6-43$^T$ (Fig. S1). This composition is consistent with peptidoglycan type A4α, which has been reported for most members of the genus *Georgenia*. Sugars from whole-cell hydrolysates found in strain 2C6-43$^T$ were rhamnose, ribose and galactose. Rhamnose and galactose have been reported for most species of genus *Georgenia* (Li *et al.*, 2007; Hamada *et al.*, 2009; Tang *et al.*, 2010), whereas ribose has been found only in strain 2C6-43$^T$. Strain 2C6-43$^T$ contained phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylinositol and minor amount of unidentified polar lipid L1 (see Fig. S2). These lipids were also detected in *G. muralis* 1A-C$^T$, *G. ruanii* YIM 004$^T$, *G. thermotolerans* TT02-04$^T$, *G. soli* CC-NMPT-T3$^T$ and *G. halophila* YIM 93316$^T$. However, the presence of minor amounts of unidentified polar lipid L1 could be useful for the differentiation of strain 2C6-43$^T$ from its closest relatives.

**Table 2.** Utilization of monochlorophenols by *Georgenia daeguensis* 2C6-43$^T$

+, Utilized; w, weak.

| Substrate | Concn (p.p.m.) | Utilization |
|---|---|---|
| 2-CP | 100 | + |
| | 200 | + |
| | 300 | + |
| | 400 | + |
| | 500 | + |
| 3-CP | 100 | w |
| | 200 | + |
| | 250 | w |
| | 300 | w |
| | 350 | w |
| 4-CP | 100 | + |
| | 150 | + |
| | 200 | + |
| | 250 | + |
| | 300 | w |

The phenotypic and phylogenetic data presented here indicate that strain 2C6-43$^T$ belongs to the genus *Georgenia*. The phylogenetic distinctiveness of 2C6-43$^T$, together with the DNA–DNA hybridization data, confirmed that this strain represents a species that is distinct from the recognized *Georgenia* species. Strain 2C6-43$^T$ can be differentiated from the five currently recognized *Georgenia* species based on several phenotypic characteristics (Table 1). Therefore, on the basis of the data presented, strain 2C6-43$^T$ should be classified within the genus *Georgenia* as a representative of a novel species, for which the name *Georgenia daeguensis* sp. nov. is proposed.

## Description of *Georgenia daeguensis* sp. nov.

*Georgenia daeguensis* (dae.gu.en′sis. N.L. fem. adj. *daeguensis* pertaining to Daegu, a city in South Korea, where the type strain was isolated).

Cells are Gram-positive, aerobic, motile, non-spore-forming rods, 0.3–0.5 μm in width and 0.5–0.8 μm in length. After 2 days incubation at 30 °C on LB agar, colonies are 1–2 mm in diameter, circular, convex, shiny, opaque and yellowish. Grows at 10–42 °C, at pH 5.0–10.0 and in the presence of 1–9 % (w/v) NaCl; no growth occurs at 4 °C, pH 11 or in the presence of 10 % NaCl; optimal growth is observed at pH 6.0–8.0 and 25–30 °C. Oxidase- and catalase-positive. Nitrate is reduced to nitrite, but nitrite is not reduced. In substrate assimilation tests, glucose, sucrose, maltose, gluconate and glycogen are utilized for growth but L-histidine, L-serine, L-proline, D-sorbitol, inositol, caprate, adipate, citrate, phenylacetate, sodium malonate, lactate, propionate, valerate, trisodium citrate and 4-hydroxybenzoate are not utilized. MK-8($H_4$) is the predominant menaquinone. The major cellular fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{15:1}$ A, iso-$C_{15:0}$, $C_{16:0}$, iso-$C_{14:0}$ and $C_{12:0}$.

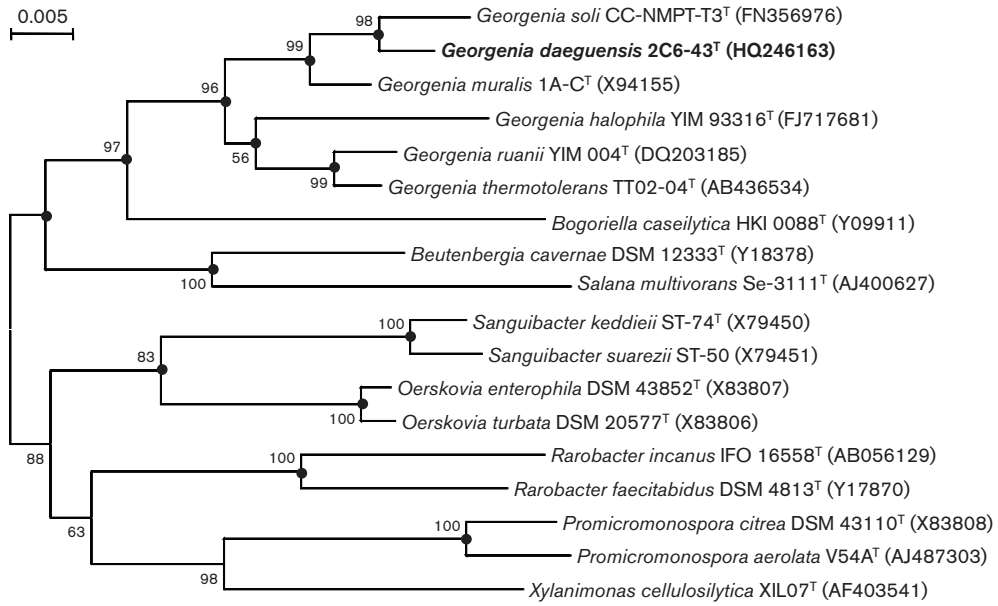


**Fig. 1.** Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain 2C6-43$^T$ among recognized *Georgenia* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony and maximum-likelihood algorithms. Bar, 0.005 substitutions per nucleotide position.

**Table 3.** Cellular fatty acid profiles of strain 2C6-43$^T$ and its closest phylogenetic neighbours in the genus *Georgenia*

Strains: 1, 2C6-43$^T$; 2, *G. soli* DSM 21838$^T$; 3, *G. muralis* DSM 14418$^T$; 4, *G. thermotolerans* DSM 21501$^T$; 5, *G. ruanii* KCTC 19029$^T$; 6, *G. halophila* DSM 21365$^T$. All data are from the present study. All strains were grown on TSA at 30 °C for 48 h prior to fatty acid analysis except *G. halophila* DSM 21365$^T$ which was grown on 10 % NaCl TSA at 37 °C for 7 days. Values are percentages of total fatty acids; −, not detected. Data below 1 % are not shown in this table.

| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Saturated | | | | | | |
| $C_{10:0}$ | 3.44 | − | 1.65 | − | 3.70 | − |
| $C_{12:0}$ | 5.55 | − | − | 3.39 | 4.19 | − |
| $C_{14:0}$ | − | − | − | − | 1.56 | − |
| $C_{15:0}$ | − | − | − | − | 2.41 | − |
| $C_{16:0}$ | 7.38 | 13.51 | 24.34 | 8.75 | 20.36 | 11.27 |
| $C_{18:0}$ | 2.30 | 7.01 | 11.68 | 3.96 | 5.86 | 6.22 |
| iso-$C_{14:0}$ | 3.80 | 3.10 | 3.62 | − | 2.04 | 1.20 |
| iso-$C_{15:0}$ | 9.48 | 8.51 | 2.48 | 8.38 | 7.90 | 4.29 |
| iso-$C_{16:0}$ | − | − | − | − | 5.07 | − |
| iso-$C_{17:0}$ | − | − | − | − | 2.04 | − |
| anteiso-$C_{13:0}$ | 2.87 | − | − | − | − | − |
| anteiso-$C_{15:0}$ | 42.96 | 52.62 | 47.29 | 65.47 | 38.36 | 66.27 |
| anteiso-$C_{17:0}$ | − | 1.63 | − | − | 1.93 | − |
| Unsaturated | | | | | | |
| $C_{12:1}$ AT 11–12 | − | 4.06 | 6.04 | − | − | 5.94 |
| anteiso-$C_{15:1}$ A | 13.33 | 9.56 | 2.90 | − | − | 3.67 |
| $C_{12:0}$ 3-OH | 1.93 | − | − | − | − | − |
| Summed feature 2* | 1.87 | − | − | − | − | − |
| Unknown | 4.27 | − | − | 2.65 | 4.58 | 1.15 |

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 contained iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH.

The type strain, 2C6-43$^T$ (=KCTC 19801$^T$=JCM 17459$^T$), was isolated from an activated sludge sample collected from an industrial wastewater treatment plant in Daegu, South Korea. The DNA G+C content of the type strain is 66.2 mol%.

## Acknowledgements

This work was supported by the GAIA Project (173-091-006), Ministry of Environment, Republic of Korea and by the WCU (World class University) Program of the National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology (R33-10076).

## References

**Altenburger, P., Kämpfer, P., Schumann, P., Vybiral, D., Lubitz, W. & Busse, H.-J. (2002).** *Georgenia muralis* gen. nov., sp. nov., a novel actinobacterium isolated from a medieval wall painting. *Int J Syst Evol Microbiol* **52**, 875–881.

**Assih, E. A., Ouattara, A. S., Thierry, S., Cayol, J.-L., Labat, M. & Macarie, H. (2002).** *Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. *Int J Syst Evol Microbiol* **52**, 559–568.

**Bae, H. S., Lee, J. M., Kim, Y. B. & Lee, S.-T. (1996).** Biodegradation of the mixtures of 4-chlorophenol and phenol by *Comamonas testosteroni* CPW301. *Biodegradation* **7**, 463–469.

**Cappuccino, J. G. & Sherman, N. (2002).** *Microbiology: a Laboratory Manual*, 6th edn. Menlo Park, CA: Benjamin/Cummings.

**Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989).** Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

**Felsenstein, J. (1981).** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

**Felsenstein, J. (1985).** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

**Felsenstein, J. (2009).** PHYLIP (phylogeny inference package), version 3.69. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

**Fitch, W. M. (1971).** Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

**Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

**Hamada, M., Tamura, T., Ishida, Y. & Suzuki, K. (2009).** *Georgenia thermotolerans* sp. nov., an actinobacterium isolated from forest soil. *Int J Syst Evol Microbiol* **59**, 1875–1879.

**Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996).** Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.

**Kämpfer, P., Arun, A. B., Busse, H.-J., Langer, S., Young, C.-C., Chen, W.-M., Schumann, P., Syed, A. A. & Rekha, P. D. (2010).** *Georgenia soli* sp. nov., isolated from iron-ore-contaminated soil in India. *Int J Syst Evol Microbiol* **60**, 1027–1030.

**Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005).** *Sphingopyxis granuli* sp. nov., a β-glucosidase-producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.

**Kimura, M. (1983).** *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

**Lee, M., Ten, L. N., Lee, H. W., Oh, H. W., Im, W. T. & Lee, S.-T. (2008).** *Sphingopyxis ginsengisoli* sp. nov., isolated from soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **58**, 2342–2347.

**Li, W.-J., Xu, P., Schumann, P., Zhang, Y.-Q., Pukall, R., Xu, L.-H., Stackebrandt, E. & Jiang, C.-L. (2007).** *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* **57**, 1424–1428.

**Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

**Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984).** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

**Moore, D. D. & Dowhan, D. (1995).** Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by F. W. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.

**Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

**Sasser, M. (1990).** Identification of bacteria through fatty acid analysis. In *Methods in Phytobacteriology*, pp. 199–204. Edited by Z. Klement, K. Rudolph & D. C. Sands. Budapest: Akademiai Kaido.

**Schleifer, K. H. (1985).** Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

**Schleifer, K. H. & Kandler, O. (1972).** Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

**Sei, K., Asano, K.-I., Tateishi, N., Mori, K., Ike, M. & Fujita, M. (1999).** Design of PCR primers and gene probes for the general detection of bacterial populations capable of degrading aromatic compounds via catechol cleavage pathways. *J Biosci Bioeng* **88**, 542–550.

**Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

**Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C., Nesme, X., Rosselló-Móra, R., Swings, J. & other authors (2002).** Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.

**Staneck, J. L. & Roberts, G. D. (1974).** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.

**Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

**Tang, S.-K., Wang, Y., Chen, Y., Lou, K., Cao, L.-L., Xu, L.-H. & Li, W.-J. (2009).** *Zhihengliuella alba* sp. nov., and emended description of the genus *Zhihengliuella*. *Int J Syst Evol Microbiol* **59**, 2025–2032.

**Tang, S.-K., Wang, Y., Lee, J.-C., Lou, K., Park, D.-J., Kim, C.-J. & Li, W.-J. (2010).** *Georgenia halophila* sp. nov., a halophilic actinobacterium isolated from a salt lake. *Int J Syst Evol Microbiol* **60**, 1317–1421.

**Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

**Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W. & Kämpfer, P. (2010).** Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* **60**, 249–266.

**Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987).** International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.