

## *Arthrobacter bambusae* sp. nov., isolated from soil of a bamboo grove

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A Gram-stain-positive, aerobic, motile by gliding, rod-shaped bacterial strain, THG-GM18<sup>T</sup>, was isolated from soil of a bamboo grove. Strain THG-GM18<sup>T</sup> was able to grow in the presence of up to 6.0% (w/v) NaCl, at 4–37 °C and at pH 7.0–10.0 in R2A medium. Based on 16S rRNA gene sequence similarity, strain THG-GM18<sup>T</sup> was closely related to species of the genus *Arthrobacter*. The most closely related strains to strain THG-GM18<sup>T</sup> are *Arthrobacter ramosus* CCM 1646<sup>T</sup> (98.5% similarity), *Arthrobacter nitroguajacolicus* G2-1<sup>T</sup> (98.4%), *Arthrobacter nicotinovorans* DSM 420<sup>T</sup> (98.2%), *Arthrobacter aurescens* DSM 20116<sup>T</sup> (98.1%) and *Arthrobacter chlorophenolicus* A6<sup>T</sup> (98.0%). Strain THG-GM18<sup>T</sup> possessed chemotaxonomic properties consistent with those of members of the genus *Arthrobacter*, such as peptidoglycan type A3 $\alpha$  (L-Lys–L-Ala–L-Thr–L-Ala), MK-9 as major menaquinone and anteiso- and iso-branched compounds (anteiso-C<sub>15:0</sub>, iso-C<sub>16:0</sub> and anteiso-C<sub>17:0</sub>) as major cellular fatty acids. The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, an unidentified phosphoglycolipid, unidentified phospholipids, unidentified aminolipids, an unidentified glycolipid and unidentified lipids. The G + C content of the genomic DNA was 61.0 mol%. The DNA–DNA relatedness values between strain THG-GM18<sup>T</sup> and its closest phylogenetic neighbours were below 26.0%. The results of physiological and biochemical tests allowed the differentiation of strain THG-GM18<sup>T</sup> from species of the genus *Arthrobacter* with validly published names. *Arthrobacter bambusae* sp. nov. is the proposed name, and the type strain is THG-GM18<sup>T</sup> (=KACC 17531<sup>T</sup>=JCM 19335<sup>T</sup>).

The genus *Arthrobacter*, the description of which was defined by Conn & Dimmick (1947) and emended by Koch *et al.* (1995), belongs to the class *Actinobacteria*. At the time of writing, the genus *Arthrobacter* comprised 70 species with validly published names. Species of the genus *Arthrobacter* have been isolated from a variety of environmental sources including soil, air, water, oil brine, plants, biofilms, cyanobacterial mats, sediment, poultry litter, cheese, human clinical specimens and animal specimens (Conn & Dimmick, 1947; Chen *et al.*, 2009; Ganzert *et al.*, 2011; Yassin *et al.*, 2011; Margesin *et al.*, 2012; Zhang *et al.*, 2012). Most members of the genus *Arthrobacter* show a rod–coccus morphological cycle and are aerobic, Gram-stain-positive and catalase-positive. Members of the genus *Arthrobacter* have high DNA G + C contents of 55–72 mol% (Conn & Dimmick, 1947;

Koch *et al.*, 1995). Fatty acids are predominantly iso- and anteiso-branched and include anteiso-C<sub>15:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, anteiso-C<sub>17:0</sub> and C<sub>16:0</sub>. The quinone system is composed of completely unsaturated or mono-saturated menaquinones with chain lengths of eight to ten isoprenoid units. The cell-wall peptidoglycan contains the diagnostic diamino acid lysine, with several variations in the interpeptide bridge conforming to peptidoglycan type A3 $\alpha$  or A4 $\alpha$ . Based on the amino acid composition of the peptidoglycan interpeptide chain, another grouping of species of the genus *Arthrobacter* was proposed by Komagata & Suzuki (1987), which dissected them into seven groups, designated Komagata/Suzuki groups I–VII. In this study, 16S rRNA gene sequence analysis indicated that strain THG-GM18<sup>T</sup> represented a species of the genus *Arthrobacter* (in Komagata/Suzuki Group II – rRNA cluster 2). Moreover, the morphological and chemotaxonomic properties indicated that THG-GM18<sup>T</sup> represents a novel species of the genus *Arthrobacter*.

Strain THG-GM18<sup>T</sup> was isolated from soil of a bamboo grove. A 1 g sample was suspended in 10 ml sterile 0.85% NaCl (w/v; saline solution). Serially diluted samples were

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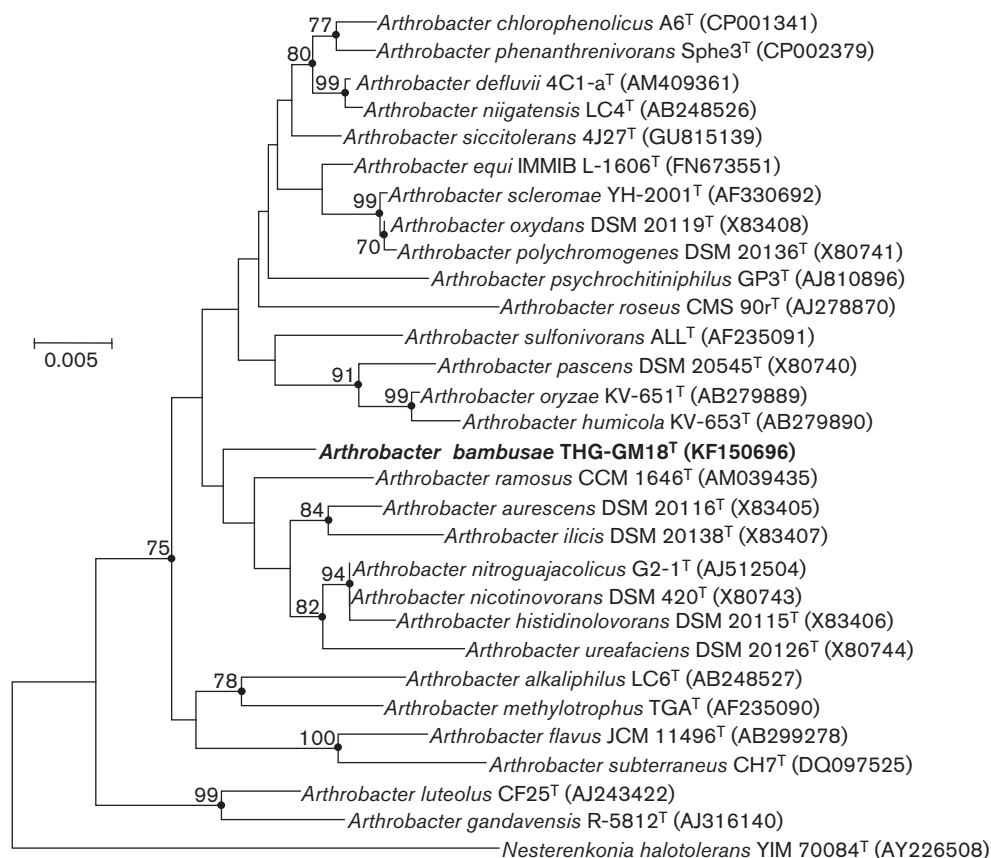
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain THG-GM18<sup>T</sup> is KF150696

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

plated onto R2A agar (BD) and were cultured on R2A agar at 28 °C for 1 week. Single colonies were purified by transferring them onto fresh R2A plates and were incubated again. One isolate, THG-GM18<sup>T</sup>, was cultured routinely on R2A agar at 28 °C and maintained in R2A medium with glycerol (25 %, v/v) at -70 °C. For sequencing of the 16S rRNA gene, genomic DNA was isolated using a Solgent genomic DNA extraction kit and the 16S rRNA gene was amplified according to the methods of Weisburg *et al.* (1991). 16S rRNA gene sequencing was performed by Solgent (Daejeon, Korea), and sequences of related taxa were retrieved from the EzTaxon-e server (Kim *et al.*, 2012) and GenBank database. Multiple alignments were performed via the CLUSTAL X program (Thompson *et al.*, 1997), followed by gap editing in the BioEdit program (Hall, 1999). The Kimura two-parameter model (Kimura, 1983) was used to calculate evolutionary distances. The neighbour-joining method (Saitou & Nei, 1987) and the maximum-likelihood method were used to reconstruct phylogenetic trees as implemented in MEGA 5.2 (Kumar *et al.*, 2008). Bootstrap values were calculated based on 1000 replications (Felsenstein, 1985).

Analysis of the 16S rRNA gene sequence (1415 bp) of strain THG-GM18<sup>T</sup> indicated that it belonged to the genus *Arthrobacter* according to the EzTaxon-e server. The most closely related strains to strain THG-GM18<sup>T</sup> were *Arthrobacter ramosus* CCM 1646<sup>T</sup> (98.5 % similarity; Jensen, 1960), *Arthrobacter nitroguajacolicus* G2-1<sup>T</sup> (98.4 %; Kotoucková *et al.*, 2004), *Arthrobacter nicotinovorans* DSM 420<sup>T</sup> (98.2 %; Kodama *et al.*, 1992), *Arthrobacter aurescens* DSM 20116<sup>T</sup> (98.1 %; Phillips, 1953) and *Arthrobacter chlorophenolicus* A6<sup>T</sup> (98.0 %; Westerberg *et al.*, 2000). The relationship between strain THG-GM18<sup>T</sup> and 28 members of the genus *Arthrobacter* was also revealed in the phylogenetic tree (Fig. 1). The phylogenetic analysis demonstrated close relationships between strain THG-GM18<sup>T</sup> and the members of rRNA cluster 2 (including *Arthrobacter histidinolovorans*, *Arthrobacter nicotinovorans*, *Arthrobacter ureafaciens*, *Arthrobacter ilicis*, *Arthrobacter aurescens* and *Arthrobacter nitroguajacolicus*; Komagata & Suzuki, 1987) (Fig. 1; Fig. S1, available in the online Supplementary Material).

The Gram reaction was determined by using a bioMérieux Gram stain kit according to the manufacturer's instructions.



**Fig. 1.** Neighbour-joining phylogenetic tree reconstructed from a comparative analysis of 16S rRNA gene sequences showing the relationships between strain THG-GM18<sup>T</sup> and related species. Filled circles at nodes indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bootstrap values (expressed as percentage of 1000 replications) >70 % are shown at branch points. Bar, 0.005 substitutions per nucleotide position.

Cell morphology was observed using cells grown in R2A broth at 28 °C for 48 h. Slides were examined using an oil immersion objective to observe cell morphology and Gram reaction with an Axio Observer D1 inverted microscope (Zeiss) at  $\times 1000$  magnification. Gliding motility was determined by using the hanging drop technique (Bernardet *et al.*, 2002). Catalase was tested by bubble production in 3 % (v/v) H<sub>2</sub>O<sub>2</sub> solution. Oxidase was tested using 1 % (w/v) *N,N,N',N'*-tetramethyl *p*-phenylenediamine reagent (Sigma). Anaerobic growth was investigated in serum bottles containing R2A broth supplemented with thioglycolate (1 g l<sup>-1</sup>) and the bottles were filled with nitrogen gas. Growth temperature was investigated on R2A agar (at 4, 10, 20, 30, 37, 40 and 45 °C). NaCl tolerance was tested in R2A broth at various NaCl concentrations (0.0–7.0 %, w/v, at intervals of 0.5 %). The pH tolerance for growth was tested in R2A broth, which was adjusted to different pH values (pH 5.0–12.0, at intervals of 0.5 pH units), after 5 days of incubation. For the pH experiments, two different buffers were used (final concentration 100 mM): acetate buffer was used for pH 5.0–6.5 and phosphate buffer was used for pH 7.0–12.0. Growth was estimated by measuring OD<sub>600</sub> using ND-1000 UV/Vis Spectrophotometer. Growth on Luria–Bertani agar (LA; Oxoid), tryptone soya agar (TSA; Oxoid), marine agar (MA; Oxoid), nutrient agar (NA; Oxoid), yeast mould agar (YM; BD) and MacConkey agar (Oxoid) was also evaluated at 28 °C after 5 days of incubation. Carbon-source utilization and enzyme activities were analysed by API 20NE, API 50CH and API ZYM according to the methods of the manufacturer (bioMérieux). Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). Biochemical tests for hydrolysis of casein, gelatin, starch, aesculin, DNA, cellulose, urea, Tween 20 and Tween 80, indole production, Voges–Proskauer reaction and H<sub>2</sub>S production from thiosulfate were performed as described by Tindall *et al.* (2007). Physiological characteristics of strain THG-GM18<sup>T</sup> are summarized in the species description and a comparison of distinctive characteristics with five related type strains is shown in Table 1. The cell morphology of strain THG-GM18<sup>T</sup> is shown in Fig. S2.

The DNA G + C content of strain THG-GM18<sup>T</sup> was determined as described by Mesbah *et al.* (1989). The DNA was hydrolysed and the nucleosides were analysed by reversed-phase HPLC [model NS-6000A, Futecs; reversed-phase column SunFire C18 (4.6  $\times$  250 mm  $\times$  5  $\mu$ m), eluted with a mixture of 0.2 M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and acetonitrile (20 : 1, v/v), flow rate 1.2 ml min<sup>-1</sup>; wavelength 270 nm]. The level of DNA–DNA relatedness was measured between strain THG-GM18<sup>T</sup> and the five reference type strains according to the method of Ezaki *et al.* (1989) using THG-GM18<sup>T</sup> photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was performed at 49.3 °C 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 were converted to percentage DNA–DNA relatedness values.

The G + C content of the genomic DNA of strain THG-GM18<sup>T</sup> was 61.0 mol%. DNA–DNA relatedness values

between strain THG-GM18<sup>T</sup>, *A. ramosus* CCM 1646<sup>T</sup> (KCTC 19355<sup>T</sup>), *A. nitroguajacolicus* G2-1<sup>T</sup> (=KACC 14581<sup>T</sup>), *A. nicotinovorans* DSM 420<sup>T</sup> (KCTC 9902<sup>T</sup>), *A. aurescens* DSM 20116<sup>T</sup> (KCTC 3378<sup>T</sup>) and *A. chlorophenolicus* A6<sup>T</sup> (=KACC 20538<sup>T</sup>) were 26.0  $\pm$  0.2, 12.1  $\pm$  2.2, 13.9  $\pm$  3.7, 11.5  $\pm$  2.8 and 13.2  $\pm$  2.1 %, respectively. These values were below 27 %, which indicated that strain THG-GM18<sup>T</sup> represented a distinct genomic species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

For fatty acid analysis, strain THG-GM18<sup>T</sup> and five reference strains were cultured on R2A agar plates at 28 °C for 2 days. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed by GC (model 6890; Hewlett Packard) using the Microbial Identification software package with the Sherlock system MIDI 6.1 and the Sherlock Aerobic Bacterial Database (TSBA 6.1) (Sasser, 1990). For analysis of the peptidoglycan structure and cell-wall sugars, freeze-dried cells of strain THG-GM18<sup>T</sup> were used. The peptidoglycan structure was determined by using hydrolysates of purified cell walls according to the protocols of Komagata & Suzuki (1987) and Busse *et al.* (1996). The amino acids and peptides were separated by two-dimensional ascending TLC on cellulose plates with the solvent systems described by Schenkel *et al.* (1995). For analysis of sugars, cell walls were hydrolysed in 0.5 M H<sub>2</sub>SO<sub>4</sub> at 100 °C for 2 h. H<sub>2</sub>SO<sub>4</sub> was removed by shaking with saturated barium hydroxide [Ba(OH)<sub>2</sub>; Duksan]. The sugars in the hydrolysate were analysed by TLC on cellulose plates according to the protocol of Stanek & Roberts (1974). For analysis of isoprenoid quinones, strain THG-GM18<sup>T</sup> was cultivated on R2A agar at 28 °C for 2 days and cells were lyophilized. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum conditions and re-extracted in *n*-hexane/water (1 : 1, v/v). The crude *n*-hexane/quinone solution was purified using Sep-Pak Silica Vac Cartridges (Waters) and then analysed by reversed-phase HPLC (model NS-6000A, Futecs), as described by Hiraishi *et al.* (1996). Polar lipids of strain THG-GM18<sup>T</sup> were analysed by two-dimensional TLC and identified as described by Minnikin *et al.* (1977, 1984).

The fatty acid profile of strain THG-GM18<sup>T</sup> mainly comprised anteiso-C<sub>15:0</sub> (57.2 %), anteiso-C<sub>17:0</sub> (16.7 %) and iso-C<sub>16:0</sub> (10.3 %) (Table 2). The cell-wall peptidoglycan of strain THG-GM18<sup>T</sup> contained lysine with the interpeptide bridge L-Ala–L-Thr–L-Ala. This polymer type is consistent with the type A3 $\alpha$  (Schleifer & Kandler, 1972) and A11.17, according to the DSMZ catalogue of strains (Schumann, 2011). Lysine is typically found in the cell wall of species of the genus *Arthrobacter* and is also the diagnostic diamino acid in the peptidoglycan of strain THG-GM18<sup>T</sup>. The peptidoglycan structure of THG-GM18<sup>T</sup> (type A11.17) was similar to those of its phylogenetic neighbours, *A. nicotinovorans* DSM 420<sup>T</sup>, *A. aurescens* DSM 20116<sup>T</sup> and *A. nitroguajacolicus* G2-1<sup>T</sup> (*Arthrobacter aurescens* group – rRNA cluster 2), but was different from those of *A. ramosus* CCM 1646<sup>T</sup> (A11.7) and *A. chlorophenolicus* A6<sup>T</sup> (A11.23).

**Table 1.** Physiological characteristics of strain THG-GM18<sup>T</sup> and related species of the genus *Arthrobacter*

Strains: 1, THG-GM18<sup>T</sup>; 2, *A. ramosus* KCTC 19355<sup>T</sup>; 3, *A. nitroguajacolicus* KACC 14581<sup>T</sup>; 4, *A. nicotinovorans* KCTC 9902<sup>T</sup>; 5, *A. aureus* KCTC 3378<sup>T</sup>; 6, *A. chlorophenolicus* KACC 20538<sup>T</sup>.

According to the API 50CH strips, all strains were positive for D-glucose and aesculin ferric citrate but negative for erythritol, D-ribose, L-xylose, L-sorbose, L-rhamnose, inositol, methyl  $\alpha$ -D-mannopyranoside, methyl  $\alpha$ -D-glucopyranoside, D-maltose, starch, glycogen, D-tagatose, gluconate and 2-ketogluconate. According to the API 20NE strips, all strains were positive for  $\beta$ -glucosidase,  $\beta$ -galactosidase, D-glucose, D-mannose, D-mannitol, D-maltose, gluconate, malate and citrate but negative for indole production, glucose acidification, arginine dihydrolase, protease and adipate. According to the API ZYM strips, all strains were positive for leucine arylamidase,  $\beta$ -galactosidase and  $\beta$ -glucosidase but negative for lipase (C14), cystine arylamidase,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase and  $\beta$ -glucuronidase. +, Positive; w, weakly positive; -, negative; ND, not detected. All data (except for DNA G+C content) were obtained in this study. DNA G+C contents of reference strains 3, 4 and 6 were obtained from Kotoucková *et al.* (2004), Kodama *et al.* (1992) and Westerberg *et al.* (2000).

Characteristic	1	2	3	4	5	6
Motility	+	+	+	+	-	+
Salt tolerance (% w/v)	6	3	6	10	ND	ND
Nitrate reduction	-	+	-	-	+	-
Growth on MacConkey agar	-	+	+	w	+	+
Hydrolysis of:						
DNA	+	-	+	+	+	+
Casein	-	-	+	w	w	+
Starch	-	+	+	+	+	-
Urea	+	+	-	-	-	-
Assimilation of:						
L-Arabinose	-	-	+	+	+	-
N-Acetylglucosamine	+	+	+	+	+	-
Caprate	-	+	-	-	+	-
Phenylacetate	-	+	-	+	+	-
Acid production from:						
Glycerol	+	+	-	-	-	-
D-Arabinose	-	+	-	-	+	-
L-Arabinose	-	-	-	+	-	-
D-Xylose	+	-	-	-	-	-
D-Adonitol	+	+	-	-	-	-
Methyl $\beta$ -D-xylose	+	-	-	+	-	-
D-Galactose	+	+	-	-	+	-
D-Fructose	+	-	+	+	-	-
D-Mannose	+	-	+	-	-	-
Dulcitol	+	+	-	-	-	-
D-Mannitol	+	+	+	+	-	-
D-Sorbitol	-	+	-	-	-	-
N-Acetylglucosamine	+	+	-	-	+	-
Amygdalin	+	-	-	-	-	-
Arbutin	+	-	-	-	-	-
Salicin	+	-	-	-	-	-
Cellobiose	+	-	-	-	-	-
Lactose	+	+	-	-	-	-

**Table 1. cont.**

Characteristic	1	2	3	4	5	6
Melibiose	+	-	-	-	-	-
Sucrose	+	-	+	+	-	-
Trehalose	+	-	-	-	-	-
Inulin	+	-	+	-	-	-
Melezitose	+	-	-	-	-	-
Raffinose	+	-	+	+	-	-
Xylitol	-	+	-	-	-	-
Gentiobiose	+	-	-	-	-	-
Turanose	+	-	-	-	-	-
D-Lyxose	+	-	-	-	-	-
D-Fucose	+	+	-	-	-	-
L-Fucose	+	-	-	-	-	-
D-Arabitol	+	+	-	-	-	-
L-Arabitol	+	+	-	-	-	-
5-Ketogluconate	+	+	-	-	-	-
Enzyme activity						
Alkaline phosphatase	-	+	+	+	+	-
Esterase (C4)	-	-	+	+	-	-
Esterase lipase (C8)	-	-	+	-	-	-
Valine arylamidase	-	-	-	+	-	-
Trypsin	-	-	+	-	+	-
Acid phosphatase	+	+	-	+	+	-
Naphthol-AS-BI-phosphohydrolase	-	+	-	-	+	-
$\alpha$ -Glucosidase	+	+	+	+	+	-
N-Acetyl- $\beta$ -glucosaminidase	-	-	-	+	+	-
$\alpha$ -Mannosidase	+	-	-	-	-	+
$\alpha$ -Fucosidase	+	-	-	-	-	-
DNA G+C content (mol%)	61.0	ND	61.9	62.4	ND	65.1

The predominant cell-wall sugars of strain THG-GM18<sup>T</sup> were galactose and glucose. Xylose, mannose and rhamnose were detected in trace amounts. The predominant menaquinone was identified as MK-9 (H<sub>2</sub>) (86.1 %). MK-7 (H<sub>2</sub>) (1.8 %), MK-8 (H<sub>2</sub>) (7.3 %) and MK-10 (H<sub>2</sub>) (4.8 %) were also detected. The major polar lipids of strain THG-GM18<sup>T</sup> were diphosphatidylglycerol (DPG), an unidentified phosphoglycolipid (PGL), an unidentified phospholipid (PL2), unidentified aminolipids (AL1-3), an unidentified glycolipid (GL) and unidentified lipids (L1-2) as shown in Fig. S3.

Based on the phenotypic, phylogenetic and genomic evidence, strain THG-GM18<sup>T</sup> represents a novel species of the genus *Arthrobacter*, for which the name *Arthrobacter bambusae* sp. nov. is proposed.

### Description of *Arthrobacter bambusae* sp. nov.

*Arthrobacter bambusae* (bam.bu'sae. N.L. fem. gen. n. *bambusae* of the bamboo genus *Bambusa*).

Cells are Gram-stain-positive, oxidase- and catalase-positive, aerobic, motile by gliding, rods which are 0.5–0.6  $\mu$ m wide and 1.0–1.6  $\mu$ m long and show a rod-coccus life cycle. Colonies on R2A agar are smooth and white. Grows on LA, TSA, MA, NA, YM and R2A agar but not on

**Table 2.** Cellular fatty acid profiles of strain THG-GM18<sup>T</sup> and related species of the genus *Arthrobacter*

Strains: 1, THG-GM18<sup>T</sup>; 2, *A. ramosus* KACC 14391<sup>T</sup>; 3, *A. nitroguajacolicus* KACC 14581<sup>T</sup>; 4, *A. nicotinovorans* KACC 20508<sup>T</sup>; 5, *A. aurescens* KACC 20528<sup>T</sup>; 6, *A. chlorophenolicus* KACC 20538<sup>T</sup>. Fatty acids amounting to <1% in all strains are not shown. Therefore, the percentages do not add up to 100%. All data are from this study. Cells of strain THG-GM18<sup>T</sup> and five reference strains were cultured on R2A agar plates for 2 days at 28 °C. TR, Trace (<1.0% of the total); —, not detected.

Fatty acid	1	2	3	4	5	6
Saturated						
C <sub>16:0</sub>	5.6	1.8	2.2	6.8	1.6	15.9
C <sub>18:0</sub>	1.9	TR	—	—	—	2.3
Unsaturated						
C <sub>14:1</sub> ω5c	TR	1.0	3.9	TR	—	TR
C <sub>18:3</sub> ω6,9,12c	TR	—	TR	TR	—	—
C <sub>20:2</sub> ω6,9c	TR	—	—	TR	—	—
Branched-chain						
iso-C <sub>14:0</sub>	1.5	2.4	2.3	1.2	3.8	TR
iso-C <sub>15:0</sub>	3.8	5.6	7.9	6.4	6.2	6.0
iso-C <sub>15:0</sub> 3-OH	TR	—	—	—	—	—
iso-C <sub>16:0</sub>	10.3	7.7	12.8	11.2	3.6	4.8
iso-C <sub>17:0</sub>	TR	TR	TR	1.1	3.1	TR
iso-C <sub>18:0</sub>	TR	—	TR	TR	—	—
anteiso-C <sub>15:0</sub>	57.2	69.2	52.4	52.4	78.7	39.9
anteiso-C <sub>17:0</sub>	16.7	8.5	10.2	15.7	3.0	9.3
anteiso-C <sub>19:0</sub>	TR	—	—	—	—	—

MacConkey agar. Growth occurs at 4–37 °C (optimum 28–30 °C), at pH 7.0–10.0 (optimum pH 7.0–8.0) and in the presence of 0.0–6.0% (w/v) NaCl. Aesculin, DNA, urea and Tween 20 are hydrolysed but casein, gelatin, starch, cellulose and Tween 80 are not. Negative for indole production, H<sub>2</sub>S production and Voges–Proskauer test. According to the API 50CH strip, positive for glycerol, D-xylose, D-adonitol, methyl β-D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, dulcitol, D-mannitol, N-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, D-lactose, melibiose, D-sucrose, trehalose, inulin, melezitose, raffinose, gentiobiose, turanose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol and 5-ketogluconate but negative for erythritol, D-arabinose, L-arabinose, D-ribose, L-xylose, L-sorbose, L-rhamnose, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, D-maltose, starch, glycogen, xylitol, D-tagatose, gluconate and 2-ketogluconate. According to the API 20NE strip, positive for urease, β-glucosidase, β-galactosidase, D-glucose, D-mannose, D-mannitol, D-maltose, gluconate, malate and citrate but negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, protease, L-arabinose, caprate, adipate and phenylacetate. In the API ZYM strip, positive for leucine arylamidase, acid phosphatase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase but negative for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase

(C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase and N-acetyl-β-glucosaminidase. MK-9 (H<sub>2</sub>) is the predominant menaquinone. Anteiso-C<sub>15:0</sub>, iso-C<sub>16:0</sub> and anteiso-C<sub>17:0</sub> are the major cellular fatty acids (>10%). The cell-wall peptidoglycan is of type A3α (L-Ala–L-Thr–L-Ala). The predominant cell-wall sugars are galactose and glucose. The diagnostic diamino acid of the peptidoglycan is lysine. The acyl type of the peptidoglycan is acetyl. The major polar lipids are diphosphatidylglycerol (DPG), an unidentified phosphoglycolipid (PGL), an unidentified phospholipid (PL2), unidentified aminolipids (AL1–3), an unidentified glycolipid (GL) and unidentified lipids (L1–2).

The type strain is THG-GM18<sup>T</sup> (=KACC 17531<sup>T</sup>=JCM 19335<sup>T</sup>), isolated from soil of a bamboo grove. The G+C content of the genomic DNA of the type strain is 61.0 mol%.

## Acknowledgements

This work was supported by a grant from Kyung Hee University, Republic of Korea in 2012 (20120115).

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