

## *Arthrobacter siccitolerans* sp. nov., a highly desiccation-tolerant, xeroprotectant-producing strain isolated from dry soil

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A novel desiccation-tolerant, xeroprotectant-producing bacterium, designated strain 4J27<sup>T</sup>, was isolated from a *Nerium oleander* rhizosphere subjected to seasonal drought in Granada, Spain. Phylogenetic analysis based on 16S rRNA gene sequencing placed the isolate within the genus *Arthrobacter*, its closest relative being *Arthrobacter phenanthrenivorans* Shep3 DSM 18606<sup>T</sup>, with which it showed 99.23% 16S rRNA gene sequence similarity. DNA–DNA hybridization measurements showed less than 25% relatedness between strain 4J27<sup>T</sup> and *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup>. The DNA base composition of strain 4J27<sup>T</sup> was 65.3 mol%. The main fatty acids were anteiso C<sub>15:0</sub>, anteiso C<sub>17:0</sub>, C<sub>16:0</sub> and iso C<sub>16:0</sub> and the major menaquinone was MK-9 (H<sub>2</sub>). The peptidoglycan type was A3 $\alpha$  with an L-Lys–L-Ser–L-Thr–L-Ala interpeptide bridge. The bacterium tested positive for catalase activity and negative for oxidase activity. Phylogenetic, chemotaxonomic and phenotypic analyses indicated that the desiccation-tolerant strain 4J27<sup>T</sup> represents a novel species within the genus *Arthrobacter*, for which the name *Arthrobacter siccitolerans* is proposed. The type strain is 4J27<sup>T</sup> (=CECT 8257<sup>T</sup>=LMG 27359<sup>T</sup>).

The genus *Arthrobacter*, first defined by Conn & Dimmick (1947), belongs to the class *Actinobacteria* and includes Gram-stain-positive coryneform bacteria with aerobic metabolism and little or no acid production from glucose. Species of the genus *Arthrobacter* contain lysine in the peptidoglycan and have a DNA G + C content ranging from 59 mol% to 66 mol% (Keddie *et al.*, 1986; Jones & Keddie, 1992). These bacteria typically take the shape of rods in younger cultures and cocci in older cultures (Keddie *et al.*, 1986), depending on their growth rate and nutritional conditions (Germida & Cassida, 1980). The transition to this coccoid-like state has been shown to require manganese (Germida & Cassida, 1980). The small coccoid-like state has been described as being the most stable form. Due to their pleomorphic and heterogeneous appearance, strains of species of the genus *Arthrobacter* were originally grouped with the *Corynebacteria* (Keddie *et al.*, 1986).

In response to changing extracellular osmolarity such as desiccation or increased salinity some micro-organisms accumulate small organic compounds (Brown, 1976; Arakawa & Timasheff, 1982). These compatible solutes act as protectants, which under laboratory conditions can also stabilize enzymes, DNA, cell membranes and even whole

cells against different kinds of stress, such as freezing, drying and heating (Brown, 1976; Yancey *et al.*, 1982; Knapp *et al.*, 1999; Manzanera *et al.* 2002, Narváez-Reinaldo *et al.*, 2010, Julca *et al.*, 2012). Our group has previously reported a new method for the isolation of desiccation-tolerant micro-organisms from dry soil using organic solvents as selective agents (Manzanera *et al.*, 2004a; Narváez-Reinaldo *et al.*, 2010). Strain 4J27<sup>T</sup> displayed remarkably high tolerance to desiccation and produced excellent xeroprotectants for the dry stabilization of proteins (lipase enzymes) and whole prokaryotic cells (*Escherichia coli* MC4100) compared with those when trehalose was used (Manzanera *et al.*, 2004b; Narváez-Reinaldo *et al.*, 2010). Among the 10 different xeroprotectants tested, the best results were observed with S4J27-D (composed of trehalose, glutamine and glucose), a synthetic mixture derived from strain 4J27<sup>T</sup> (Narváez-Reinaldo *et al.*, 2010).

Here we describe the morphological, biochemical and phylogenetic characteristics of this desiccation-tolerant strain (4J27<sup>T</sup>), isolated from dry soil and with a remarkable potential for the dry stabilization of some biomaterials. On the basis of the phylogenetic analysis of the 16S rRNA gene sequence together with physiological, chemotaxonomic and DNA–DNA hybridization analyses we demonstrate that strain 4J27<sup>T</sup> represents a novel species of the genus *Arthrobacter*.

Strain 4J27<sup>T</sup> was grown at 30 °C ( $\pm$ 3 °C) in tryptone soya agar (TSA) plates and in tryptone soya broth (TSB) or M9

Abbreviation: DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen.

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

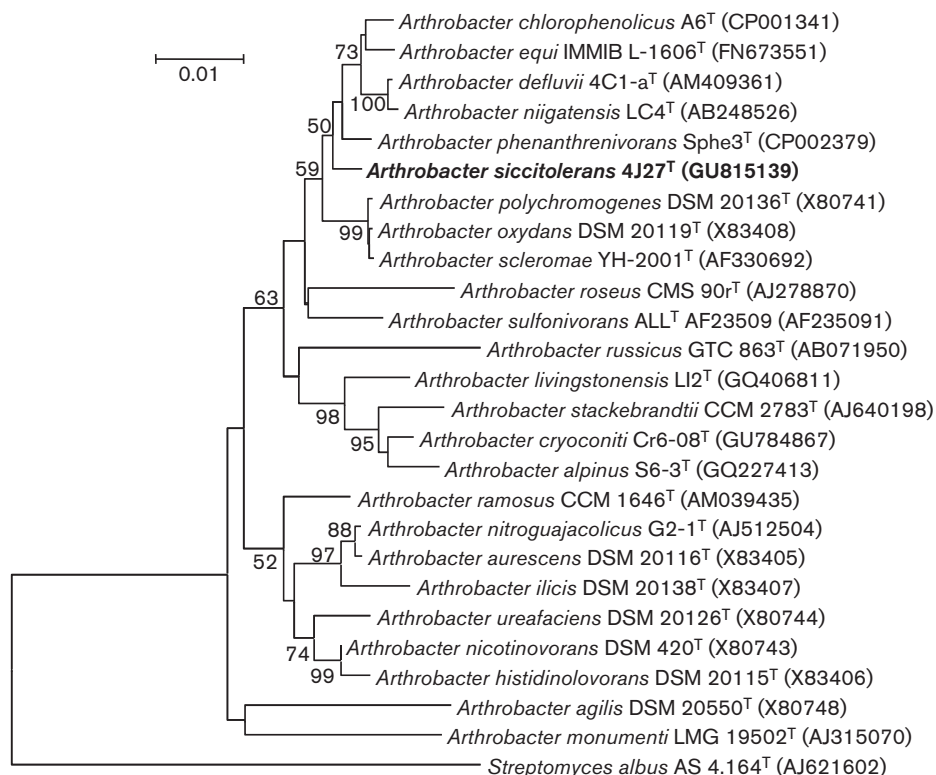
minimal medium (M6030; Sigma). *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup> was included in the study as reference.

Strain 4J27<sup>T</sup>, the object of this study, had already been assigned to the genus *Arthrobacter* by partial analysis of its 16S rRNA gene sequence (GenBank accession number GU815139; Narváez-Reinaldo *et al.*, 2010), which was compared with those in the EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net/>, Kim *et al.*, 2012). The nearly complete sequence of the 16S rRNA gene of strain 4J27<sup>T</sup> (approximately 1500 bp) was aligned with the sequences of closely related species of the genus *Arthrobacter* by using the CLUSTAL X 2 program (Larkin *et al.*, 2007). A phylogenetic tree was inferred using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Guindon & Gascuel, 2003) methods with the MEGA 5.0 software package (Tamura *et al.*, 2011). Bootstrap analysis was based on 1000 resamplings (Felsenstein, 1985). The distances were calculated according to Kimura's two-parameter model (Kimura, 1980). The resulting neighbour-joining tree obtained with Kimura's two-parameter model is shown in Fig. 1 and the maximum-likelihood tree is shown in Fig. S1, available in IJSEM Online.

The sequence corresponding to the 16S rRNA gene of strain 4J27<sup>T</sup> showed 99.23 % similarity to that of

*Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup>. Phylogenetic analysis showed that strain 4J27<sup>T</sup> clearly belongs to the genus *Arthrobacter*, and its closest relative was *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup>.

DNA–DNA hybridization was carried out at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). Cells of *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup> and strain 4J27<sup>T</sup> were disrupted by using a French pressure cell (Thermo Spectronic) and the DNA of each strain in the crude lysate were purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was conducted as described by De Ley *et al.* (1970) with the modifications described by Huss *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostat-regulated 6 × 6 multi-cell charger and a temperature controller with *in situ* temperature probe (Varian). DNA–DNA hybridization of strain 4J27<sup>T</sup> with *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup> resulted in a DNA–DNA relatedness value of 22.3 % (22.1 %), the value in parentheses being the result of measurements in duplicate. On the basis of DNA–DNA reciprocal hybridization, strain 4J27<sup>T</sup> did not belong to the species *Arthrobacter phenanthrenivorans* according to the recommendations of a threshold value of 70 % DNA–DNA



**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA sequence comparisons of strain 4J27<sup>T</sup> and its 24 closest relatives. *Streptomyces albus* AS 4.164<sup>T</sup> was used as the outgroup. The numbers at bifurcations indicate how many times each species coincided in this position as percentages and only values > 50% are shown. Bar, 0.01 changes per nucleotide position.

relatedness for the definition of bacterial species (Wayne *et al.*, 1987). Therefore strain 4J27<sup>T</sup> probably represents a novel species of the genus *Arthrobacter*.

The G + C (mol%) content of the genomic DNA of strain 4J27<sup>T</sup> was analysed at the DSMZ. The dG and dT ratio was calculated according to the method of Mesbah *et al.* (1989). Species of the genus *Arthrobacter* have previously been described as Gram-stain-positive actinobacteria with high GC content (Keddie *et al.*, 1986; Jones & Keddie, 1992), which typically have a DNA G + C content in the range of 59–66 mol% (Keddie *et al.*, 1986). The DNA G + C content of strain 4J27<sup>T</sup> was 65.3 mol%, which was within the range shown by all members of the genus *Arthrobacter* and considered to have a high GC content (Keddie *et al.*, 1986).

Chemotaxonomic analyses were carried out by the Identification Service of the DSMZ. Peptidoglycans were isolated from strain 4J27<sup>T</sup> and their structures analysed (Schleifer & Kandler, 1972). After derivatization according to the method of MacKenzie (1987) the approximate molar amino-acid ratio was determined by gas chromatography. Free amino groups within the peptidoglycan were detected by labelling with 1-fluoro-2,4-dinitrobenzene (Schleifer, 1985). The peptidoglycan of strain 4J27<sup>T</sup> was composed of Ala, Ser, Thr, Glu and Lys at a molar ratio of 2.8 : 1.2 : 1.0 : 1.0 : 1.5. Two-dimensional TLC of the partial hydrolysate (4 M HCl, 100 °C, 45 min) of the peptidoglycan revealed the presence of the peptides L-Ala-D-Glu, L-Lys-D-Ala, L-Lys-L-Ser, L-Lys-L-Ser-L-Thr, D-Ala-L-Lys-L-Ser-L-Thr, L-Ser-L-Thr and L-Ala-D-Ala. On the basis of these results it was concluded that strain 4J27<sup>T</sup> contains a type A3 $\alpha$  peptidoglycan (Schleifer & Kandler, 1972) with an L-Lys-L-Ser-L-Thr-L-Ala interpeptide bridge (A11.23 DSMZ-Catalogue of strains, 7th edition, 2001), which is found in the more closely related members of the genus *Arthrobacter*, according to the neighbour-joining phylogenetic tree, such as *Arthrobacter chlorophenicus*, *Arthrobacter oxydans*, *Arthrobacter polychromogenes*, *Arthrobacter sulfonivorans*, *Arthrobacter equi*, *Arthrobacter niigatensis*, *Arthrobacter phenanthrenivorans*, *Arthrobacter defluvi*, *Arthrobacter roseus* and *Arthrobacter scleromae* (Borodina *et al.*, 2002; Kodama *et al.*, 1992; Westerberg *et al.*, 2000; Reddy *et al.*, 2002; Huang *et al.*, 2005; Kim *et al.*, 2008; Ding *et al.*, 2009; Yassin *et al.*, 2011). Strains containing a type A3 $\alpha$  peptidoglycan make up a rather uniform group, although they do show a considerable number of different types of interpeptide bridge. Most of these strains belong to the genus *Arthrobacter* and are distinguished by strictly aerobic growth and a complete life cycle (Conn & Dimmick, 1947; Schleifer & Kandler, 1972).

Fatty-acid methyl esters were obtained from 40 mg cells of strain 4J27<sup>T</sup> scraped from Petri dishes by saponification, methylation and extraction using the methods of Miller (1982) and Kuykendall *et al.*, (1988) with minor modifications. The fatty-acid methyl-ester mixtures were separated using the Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID). The main cellular fatty acids of the highly desiccation-tolerant strain 4J27<sup>T</sup> were, from highest

to lowest, anteiso-C<sub>15:0</sub>, 41.20 %; anteiso-C<sub>17:0</sub>, 30.86 %; C<sub>16:0</sub>, 10.21 %; iso-C<sub>16:0</sub>, 6.61 %; iso-C<sub>15:0</sub>, 4.40 %; C<sub>18:0</sub>, 2.38 %; iso-C<sub>17:0</sub>, 1.79 %; iso-C<sub>14:0</sub>, 0.83 %; C<sub>14:0</sub>, 0.75 %; anteiso-C<sub>19:0</sub>, 0.61 % and iso-C<sub>18:0</sub>, 0.36 %. The fatty-acid composition of strain 4J27<sup>T</sup> was consistent with that of the genus *Arthrobacter*, with branched-chain fatty acid, anteiso-pentadecanoic acid (anteiso-C<sub>15:0</sub>) predominating (Westerberg *et al.*, 2000).

Respiratory quinones were analysed as described by Tindall (1990a; b), using TLC and UV mass spectroscopy, and found menaquinone to be the sole quinone component. Analyses of the electron-transport system (isoprenoid quinones) for strain 4J27<sup>T</sup> resulted in detection of MK9 (II-H<sub>2</sub>) 68 %; MK9 21 % and MK8 (II-H<sub>2</sub>) 11 %.

To analyse the whole cell sugars of strain 4J27<sup>T</sup>, cells were hydrolysed in 0.5 M H<sub>2</sub>SO<sub>4</sub> for 2 h at 100 °C. Sulfuric acid was removed by 20 % *N,N*-diocylmethylamine in chloroform according to the method of Whiton *et al.* (1985). Sugars in the hydrolysate were analysed by TLC on cellulose plates according to the methods of Stanek & Roberts (1974). The whole-cell sugars of the isolated strain were galactose, glucose, mannose, ribose and rhamnose.

Mobility was tested by stab-inoculating mannitol-mobility semi-solid agar (413782; Ultimed). This semi-solid agar medium enabled us to analyse the nitrate reductase activity (capacity to reduce nitrate to nitrite) and catabolism of mannitol by using Griess-Ilosvay A and B reagents. Oxidase activity was determined using 1 % w/v *N,N,N',N'*-tetramethyl-*p*-phenylenediamine and catalase activity was determined by the production of bubbles from 3 % v/v H<sub>2</sub>O<sub>2</sub>. Cells of strain 4J27<sup>T</sup> were identified as catalase-positive, oxidase-negative, nitrate reductase-negative and mannitol-positive. *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup>.

To characterize the growth of strain 4J27<sup>T</sup> at different temperatures, pH values and salinities, cultures were incubated at 150 r.p.m. in Luria-Bertani (LB) rich medium (L3152; Sigma). Cell growth was monitored at different temperatures (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C), pH (3, 5, 7, 9, 12 and 13) and NaCl concentrations (0, 0.2, 0.4, 0.6, 0.8, 1 and 1.2 M) by measuring the OD<sub>600</sub> in triplicate at 0, 12 and 24 h using a UV-160A spectrophotometer (Shimadzu). Strain 4J27<sup>T</sup> grew best at 30 °C in LB medium. It was able to grow at 37 °C and 15 °C but not at 40 °C or 10 °C. The pH range for growth was between 5 and 9 with optimum growth at pH 7. Strain 4J27<sup>T</sup> grew in NaCl concentrations ranging from 0 to 0.8 M but grew best at 0.2 M. This differed clearly from the most closely related species, *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup>, which was able to grow at 4 °C but not at pH 5.

The following API kits were used for testing, API Coryne, API 20NE and API 20E (bioMérieux,). Each test was interpreted according to the manufacturer's instructions. Biolog tests were performed to investigate which compounds the strains in question could use for respiration. A

GP2 MicroPlate (Cat. No 1014; Biolog), containing 95 different carbon compounds, was used to test for substrate oxidation. The chemistry of these plates is based on tetrazolium reduction, in response to metabolic processes such as fermentation and oxidation. Tetrazolium reduction produced formazan in a variety of colours from dark blue to deep red to orange, depending upon the original tetrazolium salt used as the substrate for the reaction. MicroPlates were inoculated and interpreted according to the manufacturer's instructions. The results were recorded after 12 h based on *A*<sub>585</sub>. Antibiotic susceptibility testing was performed using the disc-diffusion method in which the antibiotic diffuses away from the disc in two dimensions, forming a concentration gradient that inhibits the growth of bacteria and causes an inhibition zone (Piddock, 1990). The results were interpreted according to the criteria established for staphylococci in 1997 by the National Committee for Clinical Laboratory Standards (2000). At the concentrations assayed, the inhibition zone caused by streptomycin was 157 mm, rifampicin 347 mm, chloramphenicol 340 mm, kanamycin 150 mm and tetracycline 157 mm and thus it could be concluded that strain 4J27<sup>T</sup> was susceptible to all the antibiotics tested. The phenotypic differences between strain 4J27<sup>T</sup> and closely related species are summarized in Table 1 and the physiological differences between strain 4J27<sup>T</sup> and its closest relative species *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup> are summarized in Table S1.

The degree of tolerance to desiccation shown by strain 4J27<sup>T</sup> was compared with that of the previously described desiccation-tolerant bacteria *Acinetobacter calcoaceticus* PADD68 (Narváez-Reinaldo *et al.*, 2010), the desiccation-sensitive strain *Pseudomonas putida* KT2440 (Manzanera *et al.*, 2002) and the closely related *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup>. A colony of a pure culture grown for 48 h of each strain, containing 10<sup>7</sup> to 10<sup>9</sup> cells, was suspended in 1 ml M9 minimal medium. Aliquots (100 µl) were placed on sterile Petri dishes and dried under a current of sterile air for 24 h. The cells were then suspended in 1 ml sterile saline buffer, and serial dilutions of the cell suspension were plated on TSA plates before and after drying. All such procedures were conducted at room temperature. The survival rate was calculated in terms of c.f.u. ml<sup>-1</sup> after drying compared with c.f.u. ml<sup>-1</sup> before drying, expressed as a percentage. The assays were performed in triplicate accordingly to the protocol of Manzanera *et al.*, 2002. Strain 4J27<sup>T</sup> showed the highest values of desiccation tolerance (31.58% ± 6.9%), which were significantly different from those of the positive control, *Acinetobacter calcoaceticus* PADD68 (3.23% ± 0.2%) and more so from the closely related strain, *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup> (1.5% ± 0.41%). As expected, the desiccation tolerance of the negative control, *P. putida* KT2440<sup>T</sup>, was below detectable levels. Therefore the closely related species *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup> is considered to be desiccation-sensitive, due to its low degree of desiccation

tolerance, in contrast to the novel strain, which is considered to be a desiccation-tolerant strain.

On the basis of phylogenetic analysis of its 16S rRNA gene sequence, together with physiological, chemotaxonomic and DNA–DNA hybridization analyses, strain 4J27<sup>T</sup> is considered to represent a novel species of the genus *Arthrobacter*, for which the name *Arthrobacter siccitolerans* is proposed.

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

*Arthrobacter siccitolerans* (sic.ci.to'le.rans. L. adj. *siccus* dry, L. part. adj. *tolerans* tolerating; N.L. part. adj. *siccitolerans* dry-tolerating).

Cells are non-motile, non-spore-forming, Gram-positive, aerobic and rod-to-coccus-shaped. Colonies on TSA are convex, circular, cream, opaque and usually 1–2 mm in diameter within 2 days at 30 °C. Catalase-positive, oxidase-negative and nitrate-reductase-negative (no capacity to reduce nitrate to nitrite). Grows at temperatures from 15 to 35 °C, pH 5–9 and with 0–0.8 M NaCl in LB medium. The peptidoglycan type is A3α (Schleifer & Kandler, 1972), with an L-Lys–L-Ser–L-Thr–L-Ala interpeptide bridge. The major cellular fatty acids are anteiso C<sub>15:0</sub>, anteiso C<sub>17:0</sub>, C<sub>16:0</sub> and iso C<sub>16:0</sub>. The major menaquinone is MK9-(II-H<sub>2</sub>). The whole-cell sugars of the strain are galactose, glucose, mannose, ribose and rhamnose. It reduces nitrites to nitrogen. Indole and acetoin (Voges–Proskauer) production are positive. According to the results from the API CORYNE, API 20NE and API 20E strips, the following enzyme activities are detected: pirazinamidase, β-glucuronidase, β-galactosidase, α-glucosidase, β-glucosidase (aesculin), β-galactosidase (*p*-nitrophenyl-β-D-galactopyranosidase). Assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, maltose, potassium gluconate, malate, trisodium citrate, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose are positive. The following enzyme activities are not present: β-galactosidase (*o*-nitro-phenyl-β-D-galactopyranoside), arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan desaminase, gelatinase, pyrrolidonyl arylamidase, alkaline phosphatase and N-acetyl-β-glucosaminidase. Production of H<sub>2</sub>S is negative and does not use citrate. In the Biolog GP2 MicroPlates the following substrates were used for respiration: dextrin, inulin, L-arabinose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, D-arbutin, cellobiose, D-fructose, D-galactose, D-galacturonic acid, α-D-glucose, gentiobiose, lactamide, L-lactic acid, lactulose, maltose, maltotriose, D-mannitol, D-mannose, melezitose, melibiose, 3-methyl glucose, α-methyl D-mannoside, palatinose, D-ψicose, D-rafinose, L-rhamnose, D-ribose, salicin, D-sorbitol, sucrose, trehalose, turanose, xylitol, D-xylose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketovaleric acid, L-malic acid, pyruvic acid, L-alaninamide, L-alanyl glycine, glycyl-L-glutamic acid, putrescine, glycerol, adenosine, 2'-deoxy adenosine, inosine, thymidine, uridine, thymidine-5' monophosphate,

**Table 1.** Differential characteristics between strain 4J27<sup>T</sup> and the type strains of the most closely related species of the genus *Arthrobacter*

Strains: 1, 4J27<sup>T</sup>; 2, *Arthrobacter phenanthrenivorans* DSM 18606<sup>T</sup>; 3, *Arthrobacter niigatensis* IAM 15382<sup>T</sup>; 4, *Arthrobacter defluvii* DSM 18782<sup>T</sup>; 5, *Arthrobacter equi* DSM 23395<sup>T</sup>; 6, *Arthrobacter chlorophenolicus* DSM 12829<sup>T</sup>; 7, *Arthrobacter polychromogenes* DSM 20136<sup>T</sup>; 8, *Arthrobacter oxydans* DSM 20119<sup>T</sup>; 9, *Arthrobacter scleromae* JCM 12642<sup>T</sup>. Data of the reference species were taken from Kallimanis *et al.* (2009), Ding *et al.* (2009), Kim *et al.*, (2008), Yassin *et al.* (2011), Westerberg *et al.* (2000), Schippers-Lammertse *et al.* (2009), Sguros (1955), Huang *et al.* (2005) and the present study. +, Positive; −, negative; ND, not determined; CFA, cellular fatty acid.

Trait	1	2	3	4	5	6	7	8	9
Motility	−	−	−	−	−	+	−	−	−
Colony colour	Cream	Yellowish	Grey/yellow	Creamy white	Cream	Grey	Blue-green	Grey-white	White
Temperature	15–35	4–37	5–40	5–37	10–35	3–37	10–37	20–35	15–37
pH	5–9	6.5–8.5	6–11	6–10	6–9	5–9	6–11	5–9	6–9
Reduction of nitrate	+	+	+	+	−	−	+	+	+
Hydrolysis of gelatin	−	−	+	−	+	+	+	+	+
Utilization of:									
Maltose	+	−	−	−	+	+	+	+	+
D-Ribose	+	−	+	+	−	+	+	+	−
Sucrose	+	+	+	−	+	+	+	+	+
Trehalose	+	−*	+	−	+	+	−*	−*	+
D-Xylose	+	+	−	−	+	−	+	+	+
D-Alanine	−	+	ND	−	+	+	+	−*	ND
Glucose-1-phosphate	+	−*	+	ND	ND	−*	−*	−*	ND
Inulin	+	−*	ND	−	ND	−	−*	−	+
Major CFAs	antesio-C <sub>15:0</sub> antesio-C <sub>17:0</sub>	antesio-C <sub>15:0</sub> iso- C <sub>15:0</sub>	antesio-C <sub>15:0</sub> antesio- C <sub>17:0</sub>	anteiso-C <sub>15:0</sub> iso-C <sub>16:0</sub>	anteiso-C <sub>15:0</sub> iso-C <sub>15:0</sub>	anteiso-C <sub>15:0</sub> iso-C <sub>16:0</sub>	anteiso-C <sub>15:0</sub> antesio-C <sub>17:0</sub>	anteiso-C <sub>15:0</sub> anteiso-C <sub>15:0</sub>	anteiso-C <sub>15:0</sub> iso-C <sub>15:0</sub>
Major menaquinone	MK-9 (H <sub>2</sub> )	MK-8 (H <sub>2</sub> )	MK-9 (H <sub>2</sub> )	MK-9 (H <sub>2</sub> )	MK-9 (H <sub>2</sub> )	MK-9 (H <sub>2</sub> )	MK-9 (H <sub>2</sub> )	MK-9 (H <sub>2</sub> )	MK-8 (H <sub>2</sub> )
DNA G + C content (mol%)	65.3	67.5	70.8	63.5–64.4	67.0	65 ± 1	62.9	63.1	64.7

\*Data taken from the present study.

glucose-1-phosphate and D-L- $\alpha$ -glycerol phosphate. The following compounds were not used for respiration:  $\alpha$ -cyclodextrin,  $\beta$ -cyclodextrin, glycogen, mannan, Tween 40, Tween 80, amygdalin, D-arabitol, D-fructose, L-fucose, D-gluconic acid, *myo*-inositol,  $\alpha$ -D-lactose,  $\alpha$ -methyl-D-galactoside,  $\beta$ -methyl-D-galactoside,  $\alpha$ -methyl-D-glucoside,  $\beta$ -methyl-D-glucoside, palatinose, propionic acid, L-alanine, L-asparagine, L-glutamic acid, L-pyroglytamic acid and L-serine, sedoheptulose, stachyose, D-tagatose,  $\gamma$ -hydroxybutyric acid,  $\alpha$ -ketoglutaric acid, D-lactic acid methyl ester, D-malic acid, methyl pyruvate, mono-methyl succinate, succinamic acid, succinic acid, D-alanine, *N*-acetyl-L-glutamic acid, 2,3-butanediol, adenosine-5'-monophosphate, uridine-5'-monophosphate, fructose-6-phosphate and glucose-6-phosphate. Susceptible to all the antibiotics tested: streptomycin, rifampicin, chloramphenicol, kanamycin and tetracycline.

The type strain, 4J27<sup>T</sup> (=CECT 8257<sup>T</sup>=LMG 27359<sup>T</sup>), was isolated from a *Nerium oleander* rhizosphere subjected to seasonal drought in Granada, Spain. The DNA G+C content of strain 4J27<sup>T</sup> is 65.3 mol%.

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