Arthrobacter gangotriensis sp. nov. and Arthrobacter kerguelensis sp. nov. from Antarctica

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Two coryneform bacteria were isolated from a penguin rookery soil sample collected in Antarctica, near the Indian station Dakshin Gangotri (strain Lz1yT), and from sea water from Kerguelen island, Antarctica (strain KGN15T). They have morphological and chemotaxonomic properties (peptidoglycan A4 type; major menaquinones MK-8, MK-9 and MK-10; predominant fatty acids anteiso-C15:0 and anteiso-C17:0) that are characteristic of members of the genus Arthrobacter. The isolates shared 97.8% 16S rRNA gene sequence similarity to each other and were most closely related to Arthrobacter sulfureus (about 98.5% sequence similarity). DNA–DNA hybridization experiments revealed 50% relatedness between the isolates, while the levels of DNA–DNA relatedness between strains Lz1yT and KGN15T and their phylogenetic relative, A. sulfureus, were respectively 54 and 12%. Based on the above data and distinct phenotypic differences between the isolates and A. sulfureus, two novel species are proposed, Arthrobacter gangotriensis sp. nov. (type strain Lz1yT = DSM 15796 = JCM 12166) and Arthrobacter kerguelensis sp. nov. (type strain KGN15T = DSM 15797 = JCM 12165).

The genus Arthrobacter was created by Conn & Dimmick (1947) and includes bacteria that show a rod–coccus cycle, aerobic metabolism and little or no acid production from glucose, have lysine in the peptidoglycan and a DNA G+C content of 59–66 mol% (Keddie et al., 1986). The genus was later shown to include two main groups of species that differ in their peptidoglycan structural types, designated A3 and A4 (Schleifer & Kandler, 1972; Stackebrandt et al., 1983; Koch et al., 1995). The genus currently contains 34 species, of which seven, namely Arthrobacter myosores (Nand & Rao, 1972; Stackebrandt & Schumann, 2000), Arthrobacter nicotianae, Arthrobacter sulfureus, Arthrobacter uratoydans, Arthrobacter protophormiae (Stackebrandt et al., 1983), Arthrobacter rhombi (Osorio et al., 1999) and Arthrobacter creatinolyticus (Hou et al., 1998), are characterized by peptidoglycan A4. While this paper was being reviewed, Margesin et al. (2004) proposed a novel species, Arthrobacter psychrophilenolicus, which also has the peptidoglycan type A4. Until now, only three species of Arthrobacter, Arthrobacter psychrolactophilus (Loveland-Curtze et al., 1999), Arthrobacter flavus and Arthrobacter roseus (Reddy et al., 2000, 2002), that contain the A3 peptidoglycan variant have been reported from Antarctica. In the present study, we describe two novel species of the genus Arthrobacter from Antarctica, which are characterized by the A4 peptidoglycan variant.

Strain Lz1yT was isolated from a penguin rookery soil sample collected near the Indian Antarctic station, Dakshin Gangotri (70° 45′ 12″ S 11° 46′ E), and tentatively identified as A. protophormiae based on its morphology, biochemical characteristics and DNA G+C content (Shivaji et al., 1989). Strain KGN15T was isolated from a sea-water sample collected at a site located 110 km south-west of the Kerguelen Islands (50° 40′ S 68° 25′ E), Antarctica. The medium used for isolation and maintenance of Lz1yT and KGN15T was ABM agar (0.5% w/v peptone, 0.2% w/v yeast extract and 1.5% w/v agar, pH 7.2).

Morphological and growth characteristics were examined as described earlier (Reddy et al., 2000); for biochemical tests, the cultures were grown at 20°C and tests were
performed as described by Lanyi (1987) and Smibert & Krieg (1994). The ability of the cultures to utilize carbon compounds as sole carbon sources was checked with 0·5 % of each carbon compound in minimal medium [1·05 % K₂HPO₄, 0·45 % KH₂PO₄, 0·1 % (NH₄)₂SO₄, 1·5 % agar]. The sensitivity of the culture to different antibiotics was checked using antibiotic discs supplied by HiMedia pvt. Ltd (Mumbai, India). DNA was isolated and the G+C content of the DNA was determined as described by Shivaji et al. (1989). For the antibiotic sensitivity test and for the isolation of DNA, strain Lz1yT was grown in nutrient broth (0·5 % peptone, 0·3 % beef extract, 0·8 % sodium chloride, pH 7·2) and strain KGN15T in LB medium (1 % tryptone, 0·5 % yeast extract, 1 % sodium chloride, pH 7·2).

Fatty acid methyl esters, isoprenoid quinones, peptidoglycan and pigments were characterized as described previously (Reddy et al., 2002). Peptidoglycan was isolated according to the method of Komagata & Suzuki (1987). The composition and structural type of peptidoglycan (Schleifer & Kandler, 1972) was inferred on the basis of the molar ratio of amino acids. DNA–DNA hybridization was performed by the membrane filter method as described by Reddy et al. (2000). 16S rRNA genes from Lz1yT and KGN15T were amplified, sequenced (Lane, 1991; Shivaji et al., 2000) and aligned with closely related sequences retrieved from the EMBL database using CLUSTAL W (Thompson et al., 1994). Evolutionary distances were calculated by using the Kimura-2 factor (Kimura, 1980) using the DNADIST program and the phylogenetic analysis was performed using PHYLIP program (Felsenstein, 1993). A. sulfureus MTCC 1528T was used as a reference strain in studies related to morphology, biochemical tests, identification of fatty acids and DNA–DNA hybridization.

Both the strains have morphological (cells are Gram-positive, pleomorphic and non-motile) and chemotaxonomic (peptidoglycan Lys–Glu type, major menaquinones MK-8, MK-9 and MK-10, predominant fatty acids anteiso-C₁₅:₀ and anteiso-C₁₇:₀ properties that are characteristic of members of the genus Arthrobacter. Strains Lz1yT and KGN15T are closely related to A. sulfureus MTCC 1528T, with respective 16S rRNA gene similarity of 98·5 and 98·3 %, and formed a coherent clade with A. sulfureus (see tree available as supplementary material IJSEM Online). However, at the DNA–DNA level, the relatedness between A. sulfureus and each of Lz1yT and KGN15T was only 54 and 12 %, respectively. The two isolates shared 97·8 % 16S rRNA gene sequence similarity but only 50 % DNA–DNA relatedness. Further, strains Lz1yT and KGN15T exhibit a number of differences at the phenotypic level both between them and in comparison with A. sulfureus (Table 1).

Therefore, it is proposed to assign strains Lz1yT and KGN15T to novel species, with the names Arthrobacter gangotriensis sp. nov. and Arthrobacter kerguelensis sp. nov., respectively.

**Description of Arthrobacter gangotriensis sp. nov.**

Arthrobacter gangotriensis (gan.go.tri.en’sis. N.L. masc. adj. gangotriensis pertaining to the Indian Antarctic station Dakshin Gangotri).

Cells are aerobic, psychrotolerant, Gram-positive, non-motile, non-spore-forming, yellow-pigmented and exhibit a rod–coccus cycle. Grows between 4 and 30 °C. The optimum temperature and pH for growth are 22 °C and pH 7. Growth occurs in the presence of 6 % NaCl. Positive for catalase, oxidase, phosphatase, urease and gelatinase and negative for methyl red, indole and Voges–Proskauer tests, β-galactosidase, arginine dihydrolase, lysine decarboxylase and arginine decarboxylase. Does not hydrolyse aesculin, Tween 80 or starch and does not reduce nitrate to nitrite. Acid is produced from D-fructose, D-galactose and D-mannose but not from D-arabinose, D-glucose, lactose, D-mannitol, D-rhamnose, D-ribose, sucrose or D-xylene. Can utilize adonitol, D-arabinose, D-cellobiose, dulcitol, D-galactose, inulin, D-fructose, D-glucose, pyruvate, lactose, D-maltose, D-mannose, D-melibiose, D-ribose, sorbitol, sucrose, D-xylene, xylitol, L-arginine, L-asparagine, L-glucose and L-phenylalanine but not glycerol, D-mannitol, D-rhamnose, trehalose, L-alanine, L-glutamic acid and nitrofurantoin but sensitive to amikacin, ampicillin, cefoperazone, cefotaxime, ciprofloxacin, co-trimoxazole, erythromycin, chloramphenicol, kanamycin, lincomycin, lomefloxacin, norfloxacin, penicillin, roxithromycin, streptomycin, tetracycline, tobramycin and vancomycin. The G+C content of the DNA is 66 mol%. The major menaquinones MK-8, MK-9 and MK-10 are present in the ratio 1:4:5:2. The cellular fatty acids at 25 °C are anteiso-C₁₅:₀ (61·6 %), anteiso-C₁₇:₀ (5·8 %), C₁₈:₀ (9·0 %), iso-C₁₇:₀ (5·5 %), iso-C₁₆:₀ (4·0 %), iso-C₁₅:₀ (3·0 %) and C₁₆:₁ (4·2 %) (details available as supplementary material in IJSEM Online). The yellow pigment is insoluble in water but soluble in methanol and exhibits three absorption maxima, at 494, 528 and 571·5 nm. The cell-wall peptidoglycan type is Lys–Glu (variation A4z).

The type strain is Lz1yT (=DSM 15796T = JCM 12166T), isolated from penguin rookery soil.

**Description of Arthrobacter kerguelensis sp. nov.**

Arthrobacter kerguelensis (ker.gu.el.en’sis. N.L. masc. adj. kerguelensis pertaining to Kerguelen Islands, Antarctica).

Properties are very similar to those of A. gangotriensis sp. nov. (see above) with respect to morphological features, biochemical characteristics (including acid production from various carbon sources, utilization of sole carbon sources and antibiotic sensitivity), pigment characteristics and type of peptidoglycan except for the following differences. Positive for l-lysine decarboxylase, hydrolyses aesculin,
produces acid from D-xylose but not from D-galactose or D-mannose and utilizes D-rhamnose, trehalose, L-glutamic acid and L-histidine but not D-cellobiose as sole carbon sources. Resistant to norfloxacin. The G+C content of the DNA is 58 mol% and the major menaquinones MK-8, MK-9 and MK-10 are present in the ratio 4:6:1. The major cellular fatty acids at 25 °C are anteiso-C15:0 (50-0%), anteiso-C17:0 (25-4%), iso-C15:0 (6-7%), iso-C17:0 (5-1%), iso-C16:0 (4-6%) and C16:1 (3-6%) (see supplementary material).

The type strain, KGN15T (= DSM 15797T = JCM 12165T), was isolated from sea water.

**Acknowledgements**

This work was supported by the Indo-French Centre for the Promotion of Advanced Research, New Delhi, India, and the Department of Biotechnology, Government of India, New Delhi, India.

**References**


