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 Lz1y^T), and from sea water from Kerguelen island, Antarctica (strain KGN15^T). They have morphological and chemotaxonomic properties (peptidoglycan A4 α type; major menaquinones MK-8, MK-9 and MK-10; predominant fatty acids anteiso-C_{15:0} and anteiso-C_{17:0}) 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 Lz1y^T and KGN15^T 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 Lz1y^T=DSM 15796^T=JCM 12166^T) and *Arthrobacter kerguelensis* sp. nov. (type strain KGN15^T=DSM 15797^T=JCM 12165^T).

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 α and A4 α (Schleifer & Kandler, 1972; Stackebrandt *et al.*, 1983; Koch *et al.*, 1995). The genus currently contains 34 species, of which seven, namely *Arthrobacter mysorens* (Nand & Rao, 1972; Stackebrandt & Schumann, 2000), *Arthrobacter nicotianae*, *Arthrobacter sulfureus*, *Arthrobacter uratoxydans*, *Arthrobacter protophormiae* (Stackebrandt *et al.*, 1983), *Arthrobacter rhombi* (Osorio *et al.*, 1999) and *Arthrobacter creatinolyticus* (Hou *et al.*, 1998), are characterized by peptidoglycan A4 α . While this paper was being

reviewed, Margesin *et al.* (2004) proposed a novel species, *Arthrobacter psychrophenicus*, which also has the peptidoglycan type A4 α . 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 α 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 α peptidoglycan variant.

Strain Lz1y^T 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 KGN15^T 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 Lz1y^T and KGN15^T 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

Published online ahead of print on 9 July 2004 as DOI 10.1099/ijs.0.63110-0.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Lz1y^T and KGN15^T are AJ606061 and AJ606062.

A 16S rRNA gene-based phylogenetic tree and details of the fatty acid compositions of the novel strains are available as supplementary material in IJSEM Online.

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 % K_2HPO_4 , 0.45 % KH_2PO_4 , 0.1 % $(NH_4)_2SO_4$, 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 Lz1y^T was grown in nutrient broth (0.5 % peptone, 0.3 % beef extract, 0.8 % sodium chloride, pH 7.2) and strain KGN15^T 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 Lz1y^T and KGN15^T 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 1528^T 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-C_{15:0} and anteiso-C_{17:0}) properties that are characteristic of members of the genus *Arthrobacter*. Strains Lz1y^T and KGN15^T are closely related to *A. sulfureus* MTCC 1528^T, 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 Lz1y^T and KGN15^T 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 Lz1y^T and KGN15^T 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 Lz1y^T and KGN15^T 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-xylose. 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-xylose, xylitol, L-arginine, L-asparagine, L-glycine and L-phenylalanine but not glycerol, D-mannitol, D-rhamnose, trehalose, L-alanine, L-glutamic acid, L-histidine, L-leucine or tryptophan as sole carbon sources. Resistant to nalidixic acid and nitrofurantoin but sensitive to amikacin, ampicillin, cefoperazone, cefuroxime, 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-C_{15:0} (61.6 %), anteiso-C_{17:0} (5.8 %), C_{18:1} (9.0 %), iso-C_{17:0} (5.5 %), iso-C_{16:0} (4.0 %), iso-C_{15:0} (3.0 %) and C_{16:1} (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 A4x).

The type strain is Lz1y^T (=DSM 15796^T=JCM 12166^T), 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 lysine decarboxylase, hydrolyses aesculin,

Table 1. Phenotypic characteristics that differentiate *A. gangotriensis* sp. nov. Lz1y^T, *A. kerguelensis* sp. nov. KGN15^T and *A. sulfureus* MTCC 1528^T

Lz1y^T, KGN15^T and *A. sulfureus* MTCC 1528^T have the following similar characteristics. They tolerate 6% NaCl and are positive for urease and negative for starch hydrolysis and nitrate reduction. Acid is produced from D-fructose but not from D-arabinose or D-ribose. They utilize D-arabinose, dulcitol, D-galactose, inulin, D-mannose, D-melibiose, pyruvate, sorbitol, sucrose, xylitol, D-xylose, L-arginine and L-asparagine but not L-leucine as sole carbon sources. The peptidoglycan type is Lys–Glu.

Characteristic	Lz1y ^T	KGN15 ^T	<i>A. sulfureus</i> MTCC 1528 ^T
Optimum growth temperature (°C)	22	22	30
Aesculin hydrolysis	–	+	–
Casein hydrolysis	+	+	–
Lysine decarboxylase	–	+	–
Acid production from:			
D-Galactose	+	–	+
D-Mannose	+	–	–
Sucrose	–	–	+
D-Xylose	–	+	–
Utilization of sole carbon sources:			
D-Cellobiose	+	–	+
D-Mannitol	–	–	+
D-Rhamnose	–	+	+
Trehalose	–	+	+
L-Glutamic acid	–	+	+
L-Histidine	–	+	+
L-Tryptophan	–	–	+
Major menaquinones	MK-8, MK-9, MK-10	MK-8, MK-9, MK-10	MK-9, MK-10
G + C content of DNA (mol%)	66	58	64.5–66*

*Data from Stackebrandt *et al.* (1983).

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-C_{15:0} (50.0%), anteiso-C_{17:0} (25.4%), iso-C_{15:0} (6.7%), iso-C_{17:0} (5.1%), iso-C_{16:0} (4.6%) and C_{16:1} (3.6%) (see supplementary material).

The type strain, KGN15^T (=DSM 15797^T=JCM 12165^T), 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.

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