**Arthrobacter livingstonensis** sp. nov. and **Arthrobacter cryotolerans** sp. nov., salt-tolerant and psychrotolerant species from Antarctic soil

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Two novel cold-tolerant, Gram-stain-positive, motile, facultatively anaerobic bacterial strains, LI2T and LI3T, were isolated from moss-covered soil from Livingston Island, Antarctica, near the Bulgarian station St Kliment Ohridski. A rod–coccus cycle was observed for both strains. 16S rRNA gene sequence analysis revealed an affiliation to the genus *Arthrobacter*, with the highest similarity to *Arthrobacter stackebrandtii* and *Arthrobacter psychrophilinus* for strain LI2T (97.8 and 97.7 % similarity to the respective type strains) and to *Arthrobacter kerguelensis* and *Arthrobacter psychrophilicus* for strain LI3T (97.4 and 97.3 % similarity to the respective type strains). The growth temperature range was −6 to 28 °C for LI2T and −6 to 24 °C for LI3T, with an optimum at 16 °C for both strains. Growth occurred at 0–10 % (w/v) NaCl, with optimum growth at 0–1 % (w/v) for LI2T and 0.5–3 % (w/v) for LI3T. The pH range for growth was pH 4–9.5 with an optimum of pH 8 for LI2T and pH 6.5 for LI3T. The predominant fatty acids were anteiso-C15:0, C18:0 and anteiso-C17:0 for LI2T and anteiso-C15:0 and C18:0 for LI3T. Physiological and biochemical tests clearly differentiated strain LI2T from *Arthrobacter stackebrandtii* and *A. psychrophilinus* and strain LI3T from *A. kerguelensis* and *A. psychrophilicus*. Therefore, two novel species within the genus *Arthrobacter* are proposed: *Arthrobacter livingstonensis* sp. nov. (type strain LI2T = DSM 22825T = NCCB 100314T) and *Arthrobacter cryotolerans* sp. nov. (type strain LI3T = DSM 22826T = NCCB 100315T).

Species of the genus *Arthrobacter*, proposed by Conn & Dimmick (1947), have been isolated from very different sources, such as human specimens (Funke et al., 1998; Hou et al., 1998; Wauters et al., 2000; Mages et al., 2008), filtration substrates (Ding et al., 2009), the surfaces of cheese (Irlinger et al., 2005), soil and sediment (Phillips, 1953; Lee et al., 2003; Kageyama et al., 2008) as well as sewage and wastewater reservoir sediment (Kim et al., 2008; Roh et al., 2008). Some isolates are able to degrade complex organic compounds (Kodama et al., 1992; Westerberg et al., 2000; Kotoučková et al., 2004; Kallimanis et al., 2009). Over the last decade, several novel species belonging to the genus *Arthrobacter* have been isolated from cold environments such as an alpine ice cave (Margesin et al., 2004), an alpine soil (Zhang et al., 2010) and various terrestrial and aquatic habitats in the Antarctic (Reddy et al., 2000, 2002; Gupta et al., 2004; Chen et al., 2005; Wang et al., 2009).

In this study, we describe the characterization of two strains from a cold terrestrial environment in the maritime Antarctic and propose to classify them within two novel species of the genus *Arthrobacter*.

Strains LI2T and LI3T were isolated from a moss-layered soil sample collected in 2005 near the Bulgarian Antarctic station St Kliment Ohridski (62°38′ 29″ S 60°21′ 53″ W), located on Livingston Island in the South Shetland archipelago. The soil was stored at −20 °C for further microbiological investigations. For isolation, 5 g soil was mixed with 10 ml sterile 0.9 % (w/v) NaCl and shaken at 4 °C for 20 min at 150 r.p.m. Serial dilutions were made with sterile saline solution (0.9 %, w/v, NaCl), plated (0.1 ml) on a modified, synthetic BRII agar (Bunt & Rovira, 1955) and incubated at 16 °C for 7–14 days. Single colonies were then chosen for further purification. The medium used for isolation...
contained (w/v unless indicated) 0.04 % K2HPO4, 3H2O, 0.05 % (NH4)2HPO4, 0.0005 % MgSO4.7H2O, 0.01 % MgCl2, 6H2O, 0.0001 % FeCl3, 6H2O, 0.01 % CaCl2, 2H2O, 0.1 % tryptone, 0.1 % yeast extract, 0.5 % glucose, 0.03 % Na2CO3, 25 % (v/v) synthetic stone extract and 1.5 % agar, pH 8.0. The synthetic stone extract consisted of 41.5 mg NaCl, 12.5 mg AlCl3.6H2O, 5.0 mg KNO3, 80.0 mg pH 8.0. The trace metal mixture contained (w/v unless indicated) 0.04 % K2HPO4.3H2O, 1.5 % agar, pH 7.2). Growth was tested at temperatures from -6 to 28 °C for strain LI3T and up to 32 °C for strain LI2T by measuring the OD600 over 5–7 days. Salt (NaCl) tolerance was tested from 0 to 10 % (w/v) over 5–7 days, pH tolerance and optimum pH for growth were evaluated from pH 4 to 10 (in increments of 0.5 pH units) over 5–7 days. Anaerobic growth was tested on PYG agar plates (w/v; 0.1 % peptone, 0.1 % yeast extract, 0.2 % glucose, 1.5 % agar, pH 7.2) incubated under a N2/CO2 (80:20, v/v) atmosphere for 14 days. Colony characteristics were determined visually on agar plates after between 7 and 14 days of bacterial growth. Cell morphology was examined by light microscopy of cells grown for 2 and 9 days. Gram staining and flagellum and spore detection were carried out by classical procedures described by Süssmuth et al. (1999). Susceptibility to antibiotics and lysozyme was examined by a filter disc test (10 μg per disc). Acid production from carbohydrates was tested with peptone water (w/v; 1 % peptone, 0.5 % NaCl) containing solutions of various sugars (1 %, w/v) and bromothymol blue as an indicator according to Hugh & Leifson (1953). The methyl red test was performed according to Schöneder (1991). Catalase activity was determined by bubble production in a 10 % hydrogen peroxide solution. Oxidase activity was analysed with N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) as a redox indicator as described by Kovács (1956). Hydrolysis of starch and casein and the production of urease, hydrogen sulfide and indole from tryptophan were determined as described by Schröder (1991). Hydrolysis of gelatin was tested by flooding gelatin agar plates with saturated ammonium sulfate solution after incubation. To test the utilization of carbon compounds as sole carbon sources, a minimal medium was prepared (w/v unless indicated; 0.1 % NH4Cl, 0.1 % K2HPO4, 0.05 % KH2PO4, 0.02 % MgSO4.7H2O, 0.005 % CaCl2.2H2O, 0.2 % (v/v) trace metal mixture, 1.5 % agar) with 0.25 % (w/v) of each carbon compound.

For quantitative analysis of cellular fatty acid compositions, cells were grown in half-strength LB medium (pH 7.2) at 16 °C for 3 days. Extraction and analysis of polar lipids and fatty acid methyl esters was conducted according to Zink & Mangelsdorf (2004). Isoprenoid quinones were extracted using the small-scale integrated procedure of Minnikin et al. (1984). Menaquinones were analysed with a Hewlett Packard series 1050 HPLC equipped with an ODS Hypersil column and a diode-array detector. Methanol/isopropyl ether (9:2, v/v) was used as the mobile phase at a flow rate of 1.0 ml min⁻¹ and a column temperature of 30 °C (Hu et al., 1999). To determine the peptidoglycan structure, cell-wall extracts were prepared according to the method of Schleifer & Kandler (1972). After derivatization (MacKenzie, 1987), the molar ratio of the amino acids was determined by GC (Groth et al., 1996). Identified fatty acids were anteiso-C15:0 (36.4 %), C18:0 (23.0 %), anteiso-C17:0 (22.1 %), C16:0 (10.5 %), iso-C17:0 (2.3 %), iso-C15:0 (2.0 %), iso-C16:0 (1.5 %), C18:1ω9c (1.5 %) and C14:0 (0.7 %) for LI2T and anteiso-C15:0 (40.1 %), C18:0 (23.8 %), C16:0 (10.8 %), C18:2 (7.9 %), C18:1ω9c (5.8 %), iso-C15:0 (4.1 %), anteiso-C17:0ω8c (2.9 %), anteiso-C17:0ω8c (1.8 %), C20:0 (1.1 %), C18:1ω7c (0.6 %), C14:0 (0.5 %), iso-C16:0 (0.4 %) and C17:0ω3c (0.3 %) for LI3T. The polar lipids were characterized by the presence of phosphatidylglycerol only. Identified menaquinones were MK-9(H2) (69 %), MK-7(H2) (22 %) and MK-8(H2) (9 %) for strain LI2T and MK-9 (47 %), MK-10 (20 %), MK-8 (17 %), MK-7 (12 %) and MK-6 (4 %) for strain LI3T. For strain LI2T, peptidoglycan structure analyses revealed type A3w with a Lys–Thr–Ala interpeptide bridge and a substitution of the α-carboxyl group of d-glutamic acid by alanine amide, type A11L2 (DSMZ, 2001). Amino acid analyses of cell walls showed the presence of alanine, threonine and glutamic acid, with lysine as the diagnostic diamino acid. The molar ratio of Ala/Glu/Thr/Lys was 3.9:10.0:6.0:6.0. For strain LI3T, the cell-wall peptidoglycan was characterized by the presence of glutamic acid, alanine and lysine as the diagnostic diamino acid, at a molar ratio of 1.7:1.2:1.0. The peptidoglycan type was A4γ with a Lys–Glu interpeptide bridge, with glutamic acid at the N terminus (after Schleifer, 1985), type A11L4 (DSMZ, 2001).

Isolation of DNA from strains LI2T and LI3T was done using a Microbial DNA isolation kit (MoBio Laboratories) according to the manufacturer’s protocol. For 16S rRNA gene amplification, general bacterial primers 8F (Ravenschlag et al., 1999) and 1492R (Dojka et al., 1998) were used. Sequencing (by GATC Biotech, Konstanz, Germany) resulted in a 1379 bp gene product for LI2T and a 1364 bp gene product for LI3T. Alignments were used with closely related sequences obtained from GenBank using the integrated SINA alignment tool from the ARB-SILVA website (Pruesse et al., 2007) and were checked manually. The ARB program (Ludwig et al., 2004) was used for calculation of evolutionary distances and to construct a phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987; Fig. 1) using the correction of Jukes & Cantor (1969) and a termini filter that is implemented in the ARB program. To evaluate the tree
topologies, a bootstrap analysis with 1000 replications was performed. For strain LI2T, highest 16S rRNA gene sequence similarity was found to the type strains of *Arthrobacter stackebrandtii* (97.8%) and *Arthrobacter psychrochitiniphilus* (97.7%), whereas strain LI3T showed the highest sequence similarity to the type strains of *Arthrobacter kerguelensis* (97.4%) and *Arthrobacter psychrophenolicus* (97.3%). 16S rRNA gene sequence similarity between LI2T and LI3T was only 95.5%. As the 16S rRNA gene sequence similarity between the two novel strains was well below the value of 98.5% defined by Stackebrandt & Ebers (2006) as the threshold for requiring DNA–DNA hybridization experiments, we did not carry out this analysis. Determination of G+C content of DNA was done by HPLC according to the method of Mesbah et al. (1989).

Based on differences in their morphological, physiological and biochemical characteristics, strains LI2T and LI3T can...
be differentiated from the most closely related neighbours within the genus Arthrobacter (Table 1). We therefore propose the novel species Arthrobacter livingstonensis sp. nov. and Arthrobacter cryotolerans sp. nov., respectively, to accommodate the two strains.

**Description of Arthrobacter livingstonensis sp. nov.**

Arthrobacter livingstonensis (li.ving.sto.nen’sis N.L. masc. adj. livingstonensis pertaining to Livingston Island, Antarctica, the sampling location of the soil from which the type strain was isolated).

Colonies are off-white, opaque, round, slightly convex and glossy with entire margins. Cells are facultatively anaerobic, psychrotolerant, Gram-stain-positive, motile, non-spore-forming and exhibit a rod–coccus cycle. Growth occurs from −6 to 28 °C, at pH 4.0–9.5 and in the presence of 0–10 % (w/v) NaCl, with optimum growth at 16 °C, pH 8.0 and 0–1 % (w/v) NaCl. Positive for catalase, H₂S production and urease and negative for oxidase, indole production and the methyl red test. Does not hydrolyse starch. Casein hydrolysis is weak. Acid is produced from D-glucose and D-mannitol and is produced weakly from D-galactose and sucrose. No acid is produced from adonitol, L-arabinose, L-arabitol, cellobiose, dulcitol, meso-erythritol, D-fructose, L-fucose, inulin, lactose, maltose, D-mannose, melibiose, melezitose, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, trehalose or D-xylose. Can utilize

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l-arabitol, cellobiose, dulcitol, D-fructose, L-fucose, D-glucose, inulin, lactose, maltose, D-mannose, melibiose, melezitose, raffinose, D-ribose, D-sambubiose, D-salicin, sorbitol, sucrose, trehalose, D-xylene, glycerol, glycogen, L-asparagine, glycine, acetate, pyruvate and succinate as sole carbon sources, but not adonitol, meso-erythritol, formate, lactic acid or L-rhamnose. Sensitive to (10 µg per disc) penicillin, ampicillin, kanamycin, neomycin, streptomycin, erythromycin, oxytetracycline, novobiocin and rifampicin. Major fatty acids (>20% of total fatty acids) are anteisome-\(C_{15:0}\) anteisome-\(C_{17:0}\) and \(C_{18:0}\). The major menaquinone is MK-9(H2). The G+C content of the genomic DNA of the type strain is 64.7 mol%.

The type strain is LI2\(^{T}\) (=DSM 22825\(^{T}\) =NCCB 100314\(^{T}\)), isolated from a moss-covered soil from Livingston Island, South Shetland Islands, Antarctica.

**Description of Arthrobacter cryotolerans** sp. nov.

*Arthrobacter cryotolerans* (cry.o.to’ler.ans. N.L. cryo from Gr. adj. krýos cold; L. pres. part. tolerans tolerating, enduring; N.L. part. adj. cryotolerans cold-tolerating).

Colonies are yellow, opaque, round, convex and glossy with a slimy consistency and entire margins. Cells are facultatively anaerobic, psychrotolerant, Gram-stain-positive, motile, non-sporforming and exhibit a rod–coccus cycle. Growth occurs from –6 to 24 °C, at pH 4.0–9.5 and in the presence of 0–10 % (w/v) NaCl, with optimum growth at 16 °C, pH 6.5 and 0.5–3.0 % (w/v) NaCl. Positive for catalase and H2S production, and negative for oxidase, urease, indole production and the methyl red test. Does not hydrolyse starch or casein. Acid is produced weakly from D-fructose and L-rhamnose. No acid is produced from adonitol, L-arabinose, L-arabinobiose, dulcitol, meso-erythritol, L-fucose, D-glucose, D-galactose, inulin, lactose, maltose, D-mannose, D-mannitol, melibiose, melezitose, raffinose, D-ribose, salicin, D-sorbitol, sucrose, trehalose or D-xylene. Can utilize cellobiose (weakly), dulcitol (weakly), D-fructose (weakly), D-glucose, maltose (weakly), D-mannose (weakly), melezitose (weakly), raffinose, D-salicin (weakly), trehalose, glycine and glycogen as sole carbon sources, but not adonitol, L-arabitol, meso-erythritol, L-fucose, inulin, lactose, melibiose, L-rhamnose, D-ribose, sorbitol, sucrose, D-xylene, glycerol, L-asparagine, lactic acid, acetate, formate, pyruvate or succinate. Sensitive to (10 µg per disc) penicillin, ampicillin, kanamycin, neomycin, streptomycin, erythromycin, oxytetracycline, novobiocin and rifampicin. Shows weak sensitivity to lysozyme (10 µg per disc). Major fatty acids (>20% of total fatty acids) are anteisome-\(C_{15:0}\) and \(C_{18:0}\). The major menaquinone is MK-9. The G+C content of the genomic DNA of the type strain is 64.5 mol%.

The type strain is LI3\(^{T}\) (=DSM 22826\(^{T}\) =NCCB 100315\(^{T}\)), isolated from a moss-covered soil from Livingston Island, South Shetland Islands, Antarctica.

**Acknowledgements**

Our special gratitude goes to all colleagues on the Bulgarian base St Kliment Ohridski for supporting fieldwork and logistics, in particular Christo Pimpirev (Bulgarian Antarctic Institute) for leading the expedition *Livingston* 2005. Furthermore, we wish to thank Hans Hubberten and Georg Schwamborn (Alfred Wegener Institute for Polar and Marine Research) for field assistance. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) in the framework of the priority programme ‘Antarctic Research with Comparative Investigations in Arctic Ice Areas’ by a grant to D. W. (WA 1554/4).

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