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 psychrochitiniphilus 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 A. stackebrandtii and A. psychrochitiniphilus 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
NaCl, 12.5 mg AlCl₃·6 H₂O, 5.0 mg KNO₃, 80.0 mg pH 8.0. The synthetic stone extract consisted of 41.5 mg 980 contained (w/v unless indicated) 0.04 % K₂HPO₄·3 H₂O, 0.5 % peptone, 0.25 % yeast extract, 0.5 % NaCl and, if necessary, 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 % peptone, 0.1 % yeast extract, 0.5 % NaCl and 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 sugars 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 Schröder (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 (1985), type A11.26 (DSMZ, 2001). Amino acid analyses of cell walls showed the presence of alanine, threonine and glutamic acid, with lysine as the diagnostic diamino acid. 16S rRNA gene amplification, general bacterial primers 8F (Ravenschlag et al., 1999) and 1492R (Doyka 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 done 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 LI2, highest 16S rRNA gene sequence similarity was found to the type strains of *Arthrobacter stackebrandtii* (97.8%) and *Arthrobacter psychrophilinus* (97.7%), whereas strain LI3 showed the highest sequence similarity to the type strains of *Arthrobacter kerguelensis* (97.4%) and *Arthrobacter psychrophilinus* (97.3%). 16S rRNA gene sequence similarity between LI2 and LI3 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 LI2 and LI3 can

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**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strains LI2 and LI3 within the genus *Arthrobacter*. Open circles indicate branches that were also found in maximum-parsimony trees (Fitch, 1971) and shaded circles indicate branches that were also found in maximum-likelihood trees (Felsenstein, 1981); filled circles indicate branches found in both. Numbers at nodes indicate bootstrap percentages (Felsenstein, 1985) based on a neighbour-joining analysis of 1000 replications; only values $>50\%$ are shown. Bar, 0.01 substitutions per nucleotide position.
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-ramnose, D-ribose, salicin, D-sorbitol, trehalose or D-xylose. Can utilize

<table>
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<td>1–5</td>
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<td>+</td>
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<td>Peptidoglycan type</td>
<td>A3α Lys–Thr–Ala</td>
<td>A4α Lys–Glu</td>
<td>A3α</td>
<td>A3α Thr–Ala₃</td>
<td>A4α Lys–Glu</td>
<td>A3α Lys–Thr–Ala₃</td>
<td>A4α Lys–Glu</td>
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<td>Menaquinone(s)</td>
<td>9(H₂), 7(H₂), 8(H₂)</td>
<td>9(H₂), 10, 8, 7, 6</td>
<td>9(H₂)</td>
<td>9(H₂), 10(H₂), 11(H₂)</td>
<td>10, 9, 11</td>
<td>9(H₂), 8(H₂), 10(H₂)</td>
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<td>DNA G+C content (mol%)</td>
<td>64.7</td>
<td>64.5</td>
<td>58.5</td>
<td>ND</td>
<td>ND</td>
<td>61.9</td>
<td>58</td>
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</table>

Table 1. Phenotypic characteristics that differentiate isolates LI2ᵀ and LI3ᵀ from the type strains of related *Arthrobacter* species

Strains: 1, *Arthrobacter livingstonensis* sp. nov. LI2ᵀ; 2, *Arthrobacter cryotolerans* sp. nov. LI3ᵀ; 3, *A. psychrochitiniphilus* JCM 13874ᵀ (data from Wang et al., 2009); 4, *A. stackebrandtii* DSM 16005ᵀ (Tvrzová et al., 2005); 5, *A. psychrophilicus* DSM 15454ᵀ (Margesin et al., 2004); 6, *A. alpinus* S6-3ᵀ (Zhang et al., 2010); 7, *A. kerguelensis* DSM 15797ᵀ (Gupta et al., 2004). +, Positive; −, negative; w, weakly positive; ND, no data available.
a growth in the presence of 0.5–3.0 % (v/v) NaCl. Positive for catalase and H₂S production, and negative for growth at 16 °C, pH 6.5 and 0.5–3.0 % (w/v) NaCl. Does not hydrolyse starch or casein. Acid is produced from adonitol, L-arabinose, L-arabitol, cellobiose, dulcitol, D-fructose (weakly), L-fucose, D-glucose, D-mannose, melibiose, raffinose, 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 anteiso-C₁₅:₀ and C₁₈:₀. 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 LI2T (=DSM 22825T =NCCB 100314T), 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-spore-forming 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-arabitol, cellobiose, 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 and 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. Major fatty acids (>20 % of total fatty acids) are anteiso-C₁₅:₀ and C₁₈:₀. 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 LI3T (=DSM 22826T =NCCB 100315T), isolated from a moss-covered soil from Livingston Island, South Shetland Islands, Antarctica.

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