Polyphasic taxonomic study of strain CCM 2783 resulting in the description of *Arthrobacter stackebrandtii* sp. nov.

Ludmila Tvrzová,¹ Peter Schumann,² Cathrin Spröer,² Ivo Sedláček,³ Susanne Verbarq,² Reiner M. Kroppenstedt² and Zdena Páčová³

Correspondence

Ludmila Tvrzová
lida@sci.muni.cz

^{1,3}Department of Microbiology, Faculty of Science¹ and CCM – Czech Collection of Microorganisms³, Masaryk University Brno, Tvrdeho 14, 60200 Brno, Czech Republic

²DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1b, 38124 Braunschweig, Germany

Strain CCM 2783, previously classified as representing *Arthrobacter aurescens*, was subjected to a polyphasic taxonomic study. 16S rRNA gene sequence analysis and chemotaxonomic characteristics such as peptidoglycan type A3 α Lys–Ala₂, major menaquinone MK-9(H₂) and fatty acid composition confirmed assignment of the strain to the genus *Arthrobacter*. The results of phylogenetic analysis, DNA–DNA relatedness experiments and physiological and chemotaxonomic characteristics indicate that CCM 2783 differs from its nearest phylogenetic relative *Arthrobacter psychrolactophilus* and from other recognized *Arthrobacter* species. Therefore, a novel species, *Arthrobacter stackebrandtii* sp. nov., is proposed with the type strain CCM 2783^T (=DSM 16005^T).

Strain CCM 2783 was isolated from poultry litter and identified on the basis of phenotypic features as representing *Arthrobacter aurescens* (Antheunisse & Vonkeman, 1975). During the course of a taxonomic study of arthrobacters, we found that the strain differed from the type strain of *A. aurescens*. In this study, we report the taxonomic characterization of strain CCM 2783 and propose to classify it as representing a novel species of the genus *Arthrobacter*, *Arthrobacter stackebrandtii* sp. nov.

Strain CCM 2783 and the closely related type strain *Arthrobacter psychrolactophilus* DSM 15612^T were routinely cultivated on tryptone soy medium (Oxoid) at 28 °C. Standardized methods as described by Smibert & Krieg (1994) and the API CORYNE, API ZYM and Biolog systems were used for phenotypic characterization of the strains. The commercial kits were applied according to the manufacturer's instructions.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene sequence and purification of PCR products were carried out as described by Rainey *et al.*

(1996). Purified PCR products were sequenced with the CEQ DTCS Quick Start kit (Beckman Coulter) as directed in the manufacturer's protocol. The CEQ 8000 Genetic Analysis System was used for electrophoresis of the sequence reaction products.

The ae2 editor (Maidak *et al.*, 1999) was used to align the 16S rRNA gene sequence of strain CCM 2783 against those of representatives of the main bacterial lineages available from the public databases. Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms were constructed using the neighbour-joining algorithms (De Soete, 1983). Bootstrap analysis was used to evaluate the tree topology by performing 1000 resamplings (Felsenstein, 1993). DNA for DNA–DNA hybridization experiments was isolated by using a French pressure cell and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA reassociation was performed under optimal conditions (2 $\times$ SSC at 67 °C) and recorded using a Gilford 2600 spectrophotometer (Huß *et al.*, 1983; Jahnke, 1992). Automated ribotyping was carried out using the RiboPrinter microbial characterization system (Qualicon; DuPont) and *EcoRI* and *PvuII* to generate restriction fragments.

The peptidoglycan structure was determined by using total (4 M HCl, 100 °C, 16 h) and partial (4 M HCl, 100 °C, 45 min) hydrolysates of purified cell walls according to Schleifer (1985). Amino acids and peptides were separated by two-dimensional ascending TLC on cellulose plates with

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCM 2783^T is AJ640198.

An extended neighbour-joining tree based on 16S rRNA gene sequence analysis is available as a supplementary figure in IJSEM Online.

the solvent systems of Schleifer & Kandler (1972). The molar ratio of the amino acids was determined by GC as described by MacKenzie (1987). Menaquinones were extracted according to Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1996). Fatty acids were extracted and analysed following the instructions of the Microbial Identification System operating manual (MIDI, 1999).

Phylogenetic analysis based on the almost complete 16S rRNA gene sequence (1518 nt) confirmed that strain CCM 2783 belongs to the genus *Arthrobacter* and that it was distinct from *A. aurescens* (Fig. 1; an extended dendrogram is available as Supplementary Fig. A in IJSEM Online). The highest binary 16S rRNA gene sequence similarity was found to the type strain of *Arthrobacter psychrolactophilus* DSM 15612^T (98.1%). The similarity to the recently described *Arthrobacter russicus* GTC 863^T (Li *et al.*, 2004) was 95.3%. DNA–DNA relatedness between strain CCM 2783 and *A. psychrolactophilus* DSM 15612^T was only 18.7% (24.3% after repetition), which is clearly below the 70% considered to be the threshold value for the delineation of genomic species (Wayne *et al.*, 1987). The RiboPrint patterns of strain CCM 2783 (generated by *EcoRI* and *PvuII*) were different from those of the type strain of *A. psychrolactophilus* (data not shown).

Cell wall analysis revealed that lysine is the diagnostic diamino acid in the peptidoglycan of strain CCM 2783. Peptidoglycan type A3 α (according to Schleifer & Kandler, 1972) and the major menaquinone MK-9(H₂) place strain CCM 2783 within the '*Arthrobacter globiformis* group' of the genus *Arthrobacter* (Stackebrandt & Schumann, 2000). The peptidoglycan composition and menaquinone pattern allow differentiation of CCM 2783 from *A. psychrolactophilus* DSM 15612^T and related species.

The peptidoglycan of strain CCM 2783 contained an Ala₂

interpeptide bridge and the alpha-carboxyl group of D-glutamic acid at position 2 of the peptide subunit was replaced by D-alanine amide. Analysis of the peptidoglycan structure of *A. psychrolactophilus* DSM 15612^T revealed type A3 α with a Thr–Ala₃ interpeptide bridge, type A11.28 according to the DSMZ catalogue of strains (DSMZ, 2001). The presence of threonine in the interpeptide bridge linked to the lysine residue was supported by the occurrence of the characteristic stable peptide Lys–Thr in the total peptidoglycan hydrolysate. Consequently, only reduced amounts of lysine and threonine could be detected after total hydrolysis of the peptidoglycan. This is in agreement with the relatively small amount of threonine in the peptidoglycan of the *A. psychrolactophilus* type strain reported by Loveland-Curtze *et al.* (1999). The presence of threonine in the interpeptide bridge was also reported in the phylogenetic neighbours of *A. psychrolactophilus*, *Arthrobacter polychromogenes*, *Arthrobacter oxydans*, *Arthrobacter chlorophenolicus* and *Arthrobacter sulfonivorans* (Keddie *et al.*, 1986; Kodama *et al.*, 1992; Westerberg *et al.*, 2000; Borodina *et al.*, 2002) and also of *A. aurescens* (Keddie *et al.*, 1986). The Ala₂ interpeptide bridge distinguishes CCM 2783 from all these related species and also from *A. aurescens*, to which this strain was affiliated by Antheunisse & Vonkeman (1975).

Along with the major menaquinone MK-9(H₂), strain CCM 2783 contained MK-10(H₂) as a minor component and a small amount of MK-11(H₂). The strain differed in its menaquinone pattern from *A. psychrolactophilus* DSM 15612^T (Table 1) and from *A. aurescens*, which was reported to possess predominant menaquinone MK-9(H₂) and minor amounts of MK-8(H₂), MK-7(H₂) and MK-9 (Collins & Jones, 1981).

The cellular fatty acid content of strain CCM 2783 was as follows (only values >1% are reported): anteiso-C_{15:0}, 48.1%; anteiso-C_{17:0}, 14.4%; iso-C_{14:0}, 1.84%; iso-C_{15:0},

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence analysis showing the phylogenetic position of *A. stackebrandtii* sp. nov. CCM 2783^T. The sequences of members of the *Actinobacteria* were used to root the dendrogram. Bootstrap values greater than 60% are shown at branching points. Bar, 5 nucleotide substitutions per 100 nucleotides. An extended version of this tree is available as supplementary material in IJSEM Online.

Table 1. Characteristics used to distinguish *Arthrobacter stackebrandtii* sp. nov. CCM 2783^T and *A. psychrolactophilus* DSM 15612^T

+, Positive; –, negative.

Characteristic	<i>A. stackebrandtii</i> CCM 2783 ^T	<i>A. psychrolactophilus</i> DSM 15612 ^T
Growth at pH 9.1	+	–
Nitrate reduction	+	–
Urease	+	–
β -Glucuronidase	–	+
α -Glucosidase	–	+
Aesculin hydrolysis	–	+
Utilization of:		
α -Cyclodextrin	+	–
Tween 40	+	–
<i>N</i> -Acetyl-D-mannosamine	–	+
L-Arabinose	+	–
L-Fucose	+	–
D-Melezitose	–	+
Methyl α -D-galactoside	+	–
Methyl β -D-glucoside	–	+
Stachyose	+	–
L-Asparagine	+	–
Uridine	+	–
Peptidoglycan type	L-Lys–L-Ala ₂	L-Lys–L-Thr–L-Ala ₃
Menaquinones (%)	MK-9(H ₂), MK-10(H ₂), MK-11(H ₂) (62:24:5)	MK-9(H ₂), MK-8(H ₂), MK-10(H ₂) (85:6:2)

14.2 %; iso-C_{16:0}, 16.0 %; iso-C_{17:0}, 3.5 %; C_{16:0}, 1.4 %. These data are in agreement with fatty acid profiles of members of the genus *Arthrobacter*. The fatty acid profile of strain CCM 2783 differs from that of *A. psychrolactophilus* DSM 15612^T and from *A. aurescens* CCM 1649^T by higher proportions of iso-C_{15:0} and iso-C_{16:0} and a lower proportion of anteiso-C_{15:0} (Loveland-Curtze *et al.*, 1999; Kotoučková *et al.*, 2004).

Strain CCM 2783 is additionally differentiated from its closest relative *A. psychrolactophilus* DSM 15612^T on the basis of a number of physiological and biochemical characteristics (Table 1). Additional phenotypic characteristics of strain CCM 2783 are given in the species description below.

Description of *Arthrobacter stackebrandtii* sp. nov.

Arthrobacter stackebrandtii (sta.cke.brand'ti.i. N.L. gen. masc. n. *stackebrandtii* of/from Stackebrandt, named in honour of Erko Stackebrandt for his pioneering contributions to our insight into the phylogenetic structure of the suborder *Micrococcineae* and of the genus *Arthrobacter* in particular).

Cells are irregular club-shaped rods showing a rod-coccus cycle, 0.6–1 × 1–3 µm, occurring in pairs as typical V-forms. Gram-positive, non-motile and non-acid-fast,

non-spore-forming. Growth occurs at 4–30 °C. Growth occurs over a pH range 5.7–9.1 and in the presence of 5 % NaCl. Obligately aerobic. Positive for catalase, urease and pyrazinamidase. Negative for oxidase, β -glucuronidase, β -galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase and pyrrolidonyl arylamidase. Nitrate is reduced to nitrite. Nitrite is not reduced; Tween 80 and aesculin are not hydrolysed. Starch and gelatin are hydrolysed. Acid production is negative from glucose, ribose, xylose, mannitol, maltose, lactose, sucrose and glycogen. The following compounds are utilized (Biolog system): α -cyclodextrin, dextrin, Tween 40, L-arabinose, D-arabitol, arbutin, D-cellobiose, D-fructose, L-fucose, D-galactose, α -D-glucose, α -D-lactose, lactulose, maltose, maltotriose, D-mannose, D-melibiose, methyl α -D-galactoside, methyl β -D-galactoside, palatinose, D-psicose, D-raffinose, D-ribose, salicin, stachyose, sucrose, D-trehalose, turanose, D-xylose, β -ketovaleic acid, methyl pyruvate, pyruvic acid, L-asparagine, glycerol, adenosine, inosine, thymidine and uridine. Negative reactions (Biolog) were observed with β -cyclodextrin, glycogen, inulin, mannan, Tween 80, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, amygdalin, D-galacturonic acid, gentiobiose, D-gluconic acid, *meso*-inositol, D-melezitose, 3-methyl glucose, methyl α -D-glucoside, methyl β -D-glucoside, methyl α -D-mannoside, L-rhamnose, sedoheptulosan, D-sorbitol, xylitol, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α -ketoglutaric acid, lactamide, D-lactic acid methyl ester,

D-malic acid, monomethyl succinate, propionic acid, succinamic acid, succinic acid, *N*-acetylglutamic acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-glutamic acid, glycyl L-glutamic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, adenosine 5'-monophosphate, uridine 5'-monophosphate, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate and DL-glycerophosphate.

The type strain, CCM 2783^T (= DSM 16005^T), was isolated from poultry litter.

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