Microlunatus soli sp. nov., isolated from soil

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A Gram-stain-positive, coccoid, non-endospore-forming actinobacterium (strain CC-12602^T) was isolated from a spawn used for growing the edible mushroom *Agaricus brasiliensis* in the laboratory. On the basis of 16S rRNA gene sequence analysis, strain CC-12602^T was shown to belong to the genus *Microlunatus* and was related most closely to the type strains of *Microlunatus ginsengisoli* (96.1 % similarity), *M. phosphovorius* (95.9 %), *M. panaciterrae* (95.8 %) and *M. aurantiacus* (95.5 %). The quinone system comprised menaquinone MK-9(H₄) as the major component and the polyamine pattern consisted of spermidine and spermine as major compounds. The predominant polar lipids were phosphatidylglycerol and unknown phospholipid PL3. Moderate amounts of diphosphatidylglycerol, an unknown glycolipid and three unknown phospholipids and minor amounts of an unknown phospholipid and a polar lipid were detected. The peptidoglycan type was A3γ', based on LL-2,6-diaminopimelic acid with an interpeptide bridge consisting of a single glycine residue and a second glycine residue at position 1 of the peptide subunit. Peptidoglycan structure and major fatty acids (anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{15:0}) supported the affiliation of strain CC-12602^T to the genus *Microlunatus*. The results of physiological and biochemical tests allowed strain CC-12602^T to be differentiated phenotypically from recognized *Microlunatus* species. Strain CC-12602^T is therefore considered to represent a novel species of the genus *Microlunatus*, for which the name *Microlunatus soli* sp. nov. is proposed. The type strain is CC-12602^T (=DSM 21800^T =CCM 7685^T).

The genus *Microlunatus* was proposed by Nakamura *et al.* (1995) and, at the time of writing, comprises four recognized species, *Microlunatus phosphovorius* (Nakamura *et al.*, 1995), *M. ginsengisoli* (Cui *et al.*, 2007), *M. aurantiacus* (Wang *et al.*, 2008) and *M. panaciterrae* (An *et al.*, 2008). These species possess LL-2,6-diaminopimelic acid (LL-Dpm) in the cell-wall peptidoglycan, MK-9(H₄) as the predominant menaquinone and anteiso-C_{15:0}, iso-C_{15:0} and iso-C_{16:0} as major fatty acids.

Abbreviation: LL-Dpm, LL-2,6-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-12602^T is FJ807672.

The polar lipid profile of strain CC-12602^T after two-dimensional TLC and detection with molybdatophosphoric acid is available as supplementary material with the online version of this paper.

A *Microlunatus*-like strain, CC-12602^T, was isolated from a spawn used for growing the edible mushroom *Agaricus brasiliensis* in the laboratory and was maintained on nutrient agar (Hi-Media) after incubation at 30 °C for 48 h. The strain was preserved at –80 °C in nutrient broth (Hi-Media) with 20 % (v/v) glycerol or by lyophilization.

Morphological properties, Gram-staining and cell morphology were observed by phase-contrast microscopy as described by Kämpfer & Kroppenstedt (2004).

The 16S rRNA gene sequence of strain CC-12602^T was determined and analysed as described previously (Young *et al.*, 2005). Multiple sequence alignment and analysis of the data were performed by using the software package MEGA version 4 (Tamura *et al.*, 2007). Genetic distance calculations (distance options according to Kimura's two-parameter model) and clustering with the neighbour-

joining method (Fig. 1) and maximum-parsimony method (not shown) were performed. Bootstrap analysis was based on 1000 replications. The 16S rRNA gene sequence of strain CC-12602^T was a continuous stretch of 1475 bp. 16S rRNA gene sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain CC-12602^T were *M. ginsengisoli* Gsoil 633^T (96.1%), *M. phosphovor*us DSM 10555^T (95.9%), *M. panaciterrae* Gsoil 954^T (95.8%) and *M. aurantiacus* YIM 45721^T (95.5%).

Cells cultivated in trypticase soy yeast extract medium (medium 92; <http://www.dsmz.de>) at 28 °C were used for isolation of the peptidoglycan after disruption of cells by shaking with glass beads and subsequent trypsin digestion, according to the method of Schleifer (1985). Amino acids and peptides in the cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates by using the solvent systems described by Schleifer (1985). Molar ratios of amino acids were determined by GC (GC 14A; Shimadzu) and GC-MS (320-MS Quadrupole GC/MS; Varian) of *N*-heptafluorobutyryl amino acid isobutyl esters (MacKenzie, 1987; Groth *et al.*, 1996). The hydrolysate (4 M HCl, 100 °C, 16 h) of the purified peptidoglycan of strain CC-12602^T contained the amino acids LL-Dpm, alanine, glycine and glutamic acid in a molar ratio of approximately 0.8:0.9:2.2:1.0. The peptides Gly-D-Glu, D-Ala-Gly and LL-Dpm-D-Ala were detected in the partial hydrolysate (4 M HCl, 100 °C, 0.75 h) of the peptidoglycan. From these data it was concluded that the peptidoglycan of strain CC-12602^T is of type A3γ', based on LL-Dpm with an interpeptide bridge consisting of a single glycine residue and a second glycine residue at position 1 of the peptide subunit (Schleifer & Kandler, 1972). Peptidoglycan type A3γ' was also detected in *M.*

*phosphovor*us, the type species of the genus (Schumann *et al.*, 1997).

For quinone and polar lipid analyses, cells were grown for 48 h at 28 °C in PYE (0.3% peptone from casein, 0.3% yeast extract, pH 7.2). Polyamines were extracted and analysed from PYE-grown biomass, which had been harvested at late-logarithmic growth phase, as described by Altenburger *et al.* (1997). Analyses of quinones and polar lipids were carried out according to Tindall (1990a, b) and Altenburger *et al.* (1996). HPLC conditions used for analysis of quinones and polyamines were as described by Stolz *et al.* (2007). The polyamine pattern was composed of the major compounds spermidine [5.0 μmol (g dry weight)⁻¹] and spermine [4.3 μmol (g dry weight)⁻¹]. In addition, small amounts [<0.2 μmol (g dry weight)⁻¹] of putrescine and cadaverine were detected. This polyamine pattern is in accordance with that of *M. phosphovor*us and other representatives of the family *Propionibacteriaceae*, including members of the genera *Propionibacterium*, *Propioniferax*, *Friedmanniella*, *Luteococcus* and *Tessaracoccus* (Hamana, 1995; Busse & Schumann, 1999; Kämpfer *et al.*, 2009). The quinone system of strain CC-12602^T comprised menaquinone MK-9(H₄) (92%) and MK-8(H₄) (8%). This quinone pattern is similar to those reported for recognized species of the genus *Micrococcus* (Nakamura *et al.*, 1995; Cui *et al.*, 2007; Wang *et al.*, 2008; An *et al.*, 2008). The polar lipid profile consisted of phosphatidylglycerol and unknown phospholipid PL3 as major components, moderate amounts of diphosphatidylglycerol, three unknown phospholipids and an unknown glycolipid, and minor amounts of another phospholipid and a polar lipid not containing a phosphate group, an amino group or a sugar moiety (Supplementary Fig. S1, available in IJSEM Online). Unlike *M. aurantiacus*

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses), showing the position of strain CC-12602^T. Multiple alignment, distance calculations (distance options according to Kimura's two-parameter model) and clustering with the neighbour-joining method were performed by using the software package MEGA version 4 (Tamura *et al.* 2007). Bootstrap values based on 1000 replications are given as percentages at branch points. Bar, 0.02 substitutions per nucleotide position.

(Wang *et al.*, 2008), neither phosphatidylethanolamine nor phosphatidylinositol was detected.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996), except that strains were cultivated on

Table 1. Major fatty acids of strain CC-12602^T and the type strains of recognized *Microlunatus* species

Strains: 1, CC-12602^T; 2, *M. panaciterrae* Gsoil 954^T; 3, *M. ginsengisoli* DSM 17942^T; 4, *M. phosphovorius* DSM 10555^T; 5, *M. aurantiacus* DSM 18424^T. Results are percentages of total fatty acids. Data are from the present study unless indicated otherwise. All strains were grown on R2A agar for 5 days at 30 °C. ND, Not detected. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* isomers are indicated by the suffix *c*.

Fatty acid	1*	2	3	4	5
Saturated					
C _{14:0}				0.5 (0.5)†	
C _{15:0}	1.0	(0.8)	0.9	0.3 (1.9)	1.9
C _{16:0}	0.6		1.4	2.4 (0.7)	2.8
C _{17:0}		(2.1)	1.6 (0.8)		
Unsaturated					
C _{16:1} ω 11 <i>c</i>	1.2				
C _{17:1} ω 8 <i>c</i>		(8.8)			
C _{17:1} ω 6 <i>c</i>	3.0				
C _{18:1} ω 7 <i>c</i>	32.8				
C _{20:4} ω 6,9,12,15 <i>c</i>					10.0
Branched-chain					
anteiso-C _{13:0}				1.1 (1.0)	0.3
iso-C _{14:0}			4.5 (8.2)	5.5 (8.9)	4.9
iso-C _{15:0}	8.6	(17.2)	21.0 (13.2)	22.5 (17.1)	4.8
iso-C _{15:1} G			ND (2.1)		
anteiso-C _{15:0}	18.3	(53.1)	39.9 (48.8)	32.2 (46.0)	46.6
anteiso-C _{15:1} A		(2.6)	ND (0.9)		
iso-C _{16:0}	15.3	(6.7)	23.7	23.5 (15.3)	11.5
iso-C _{16:1} H				ND (1.0)	
anteiso-C _{16:0}					
iso-C _{17:0}	2.6	(2.7)	1.7	6.8 (2.2)	
anteiso-C _{17:0}	8.6	(3.1)	3.9 (2.6)	2.3 (1.2)	
Hydroxy					
C _{14:0} 2-OH	1.3				
iso-C _{14:0} 3-OH	4.3			2.7 (3.9)	6.4
Summed feature 3‡					2.4
Summed feature 4‡		(3.1)		0.5 (1.3)	2.2

*It must be noted that this fatty acid profile is unusual for a member of the genus *Microlunatus*; however, it has been confirmed by analysis at the DSMZ (R. Pukall, personal communication). The deviations of the equivalent chain lengths for C_{16:1} ω 11*c*, iso-C_{14:0} 3-OH, C_{14:0} 2-OH, C_{17:1} ω 9*c* and C_{18:1} ω 7*c* are relatively high (0.004–0.008). Therefore, we cannot exclude the possibility that fatty acids other than those assigned not available in the MIDI database are present.

†Data in parentheses are from An *et al.* (2008).

‡Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprised C_{16:1} ω 7*c* and/or iso-C_{15:0} 2-OH; summed feature 4 comprised iso-C_{17:1} I/anteiso-C_{17:1} B.

R2A agar for 5 days at 30 °C prior to analysis. The fatty acid profile of strain CC-12602^T was similar to those of *M. ginsengisoli* and *M. panaciterrae*, although significant differences were also observed (Table 1). Interestingly, strain CC-12602^T contained large amounts of C_{18:1} ω 7*c* and small amounts of other fatty acids, not found in the four recognized *Microlunatus* species. Major fatty acids anteiso-C_{15:0} and iso-C_{16:0} typical of the genus *Microlunatus* were also found in strain CC-12602^T.

Results of comparative physiological characterization under identical test conditions are given in Table 2 and in the species description below, with methods as described by Kämpfer *et al.* (1991). DNA–DNA hybridization experiments were not performed between strain CC-12602^T and the type strains of recognized *Microlunatus* species because of the low levels of 16S rRNA gene sequence similarity (<97%) between these strains. Differences in the fatty acid profiles and physiological test results between strain CC-12602^T and the type strains of

Table 2. Differential carbon source assimilation characteristics of strain CC-12602^T and the type strains of recognized *Microlunatus* species

Strains: 1, CC-12602^T; 2, *M. aurantiacus* DSM 18424^T; 3, *M. phosphovorius* DSM 10555^T; 4, *M. ginsengisoli* DSM 17942^T; 5, *M. panaciterrae* Gsoil 954^T (data from An *et al.*, 2008). Data are from the present study unless indicated. All strains were positive for assimilation of L-arabinose*, D-glucose*, D-mannose*, maltose, melibiose*, L-rhamnose, D-ribose, salicin and D-sorbitol and negative for assimilation of D-gluconate, adipate, itaconate, L-alanine, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. +, Positive; (+), weakly positive; –, negative.

Assimilation of:	1	2	3	4	5
N-Acetyl-D-glucosamine	+	+	+	+	–
Arbutin	+	–	+	+	–
Cellobiose	+	+	+	+	–
D-Fructose	+	+	+	+	–
D-Galactose	+	(+)*	+	+	–
Sucrose	+	+	–	+	+
Salicin	+	–	+	+	+
Trehalose	+	+	+	+	–
D-Xylose	+	+	+	+	–
Adonitol	+	–	+	+	–
myo-Inositol	+	+	+	+	–
Maltitol	+	+	+	+	–
D-Mannitol	+	+	+	+	–
Acetate	+	(+)*	–	(+)	–
Propionate	+	–*	–	–	–
L-Malate	(+)	(+)*	+	+	–
L-Histidine	(+)	–	–	(+)	–
L-Proline	(+)	–	–	(+)	–
L-Serine	(+)	–*	–	–	–

*In agreement with results described by An *et al.* (2008).

recognized *Micrococcus* species (Table 2) clearly suggest that it represents a novel species of the genus *Micrococcus*, for which the name *Micrococcus soli* sp. nov. is proposed.

Description of *Micrococcus soli* sp. nov.

Micrococcus soli (so'li. L. neut. gen. n. *soli* of soil, the source of the type strain).

Cells are coccoid, about 1.0–1.5 µm in diameter. Gram-stain-positive, oxidase-positive and catalase-positive, showing an aerobic respiratory metabolism. Good growth occurs after 3 days of incubation on R2A agar and nutrient agar at 25–30 °C. The peptidoglycan type is A3γ' based on LL-Dpm. The polyamine pattern is composed of spermidine and spermine as major compounds. The quinone system comprises menaquinone MK-9(H₄) as the major component. The polar lipid profile consists of phosphatidylglycerol and unknown phospholipid PL3 as major components, moderate amounts of diphosphatidylglycerol, three unknown phospholipids and an unknown glycolipid and minor amounts of another phospholipid and a polar lipid not containing a phosphate group, an amino group or a sugar moiety. Phosphatidylethanolamine and phosphatidylinositol are not detected. Major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and C_{18:1}ω7c (Table 1). A small amount of C_{14:0} 2-OH is also found. Details of carbon source utilization (differential characteristics under identical conditions) are given in Table 2. Able to utilize numerous sugar compounds.

The type strain, CC-12602^T (=DSM 21800^T =CCM 7685^T), was isolated from a spawn used for growing the edible mushroom *Agaricus brasiliensis* in the laboratory.

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