Microbacterium arthrosphaerae sp. nov., isolated from the faeces of the pill millipede *Arthrosphaera magna* Attems

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A Gram-reaction-positive, rod-shaped bacterium, designated strain CC-VM-Y^T, was isolated from the faeces of the pill millipede *Arthrosphaera magna* Attems from India and was subsequently studied to determine its taxonomic position. Based on 16S rRNA gene sequence similarities, the isolate clearly grouped with members of the genus *Microbacterium*. On the basis of pairwise comparisons of the 16S rRNA gene sequences, strain CC-VM-Y^T was most closely related to *Microbacterium insulae* DS-66^T (98%), *Microbacterium hydrocarbonoxydans* DSM 160809^T (97.8%) and *Microbacterium hominis* NBRC 15708^T (97.9%). The peptidoglycan contained the amino acids ornithine (Orn), alanine (Ala), glycine (Gly), homoserine (Hsr) and glutamic acid (Glu) in an approximate molar ratio of 1.0:0.8:2.2:0.8:0.3. In addition, substantial amounts of *threo*-3-hydroxy glutamic acid (Hyg) were detected, which is characteristic of peptidoglycan type B2β. The acyl type of the peptidoglycan was glycolyl. The menaquinones of strain CC-VM-Y^T were MK-13 (72%), MK-12 (25%) and MK-11 (3%). The polar lipids consisted of phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid and one unknown glycolipid. The fatty acid profile comprised anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0} as the major fatty acids, which was congruent with fatty acid profiles of other members of the genus *Microbacterium*. The results of physiological and biochemical tests as well as DNA–DNA hybridizations with the most closely related species, *M. insulae*, *M. hydrocarbonoxydans* and *M. hominis*, revealed clear phenotypic and genotypic differences between strain CC-VM-Y^T and other members of the genus *Microbacterium*. Based on these results, strain CC-VM-Y^T (=DSM 22421^T =CCM 7681^T) represents a new species of the genus *Microbacterium*, for which the name *Microbacterium arthrosphaerae* sp. nov. is proposed.

Abbreviations: DPG, diphosphatidylglycerol; GL, glycolipid; Hyg, *threo*-3-hydroxy glutamic acid; PG, phosphatidylglycerol; PL, phospholipid; pNA, para-nitroanilide; pNP, para-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-VM-Y^T is FN870023.

One supplementary figure and one supplementary table are available with the online version of this paper.

The genus *Microbacterium* was proposed by Orla-Jensen (1919) and the genus description was emended by Collins *et al.* (1983) and later by Takeuchi & Hatano (1998), who proposed the unification of the genera *Microbacterium* and *Aureobacterium*. At the time of writing, the genus comprised >70 species with validly published names. Microbacteria are typically Gram-positive, non-spore-forming rods, which often produce yellow-pigmented

colonies. Members of the genus *Microbacterium* have been isolated from a wide variety of environmental sources, including soil, water and plants as well as from steep liquor, milk products and clinical specimens.

Here, we describe the phenotypic and genotypic properties of a novel strain isolated from the faeces of the pill millipede *Arthrosphaera magna* Attems from India.

Strain CC-VM-Y^T was obtained from faeces of a pill millipede by culturing on nutrient agar (Oxoid) at 30 °C for 48h. The isolate was preserved at –80 °C in nutrient broth (NB; Oxoid) with 20 % (v/v) glycerol or by lyophilization.

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

The 16S rRNA gene sequence was determined as described by Young *et al.* (2005). Multiple sequence alignment and analysis of the data were performed using the software package MEGA version 4 (Tamura *et al.*, 2007). Genetic distance calculations (distance options according to the

Kimura-2 model) and clustering using the neighbour-joining (Fig. 1) and maximum-parsimony (results not shown) methods were performed by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain CC-VM-Y^T was a continuous stretch of 1339 bp. On the basis of pairwise comparisons of 16S rRNA gene sequences using the EzTaxon program (Chun *et al.*, 2007), strain CC-VM-Y^T showed 98.0, 97.8 and 97.9 % sequence similarity to *Microbacterium insulae* DS-66^T, *Microbacterium hydrocarbonoxydans* DSM 160809^T and *Microbacterium hominis* NBRC 15708^T, respectively. 16S rRNA sequence similarities to all other species of the genus *Microbacterium* were <97.0 %. The peptidoglycan of strain CC-VM-Y^T was analysed by using the methods of Schleifer & Kandler (1972), Schleifer (1985), MacKenzie (1987) and Groth *et al.* (1996). The acyl type of the peptidoglycan was determined according to Uchida *et al.* (1999). Polar lipids and menaquinones were studied by using the methods of Groth *et al.* (1996). Amino acids were analysed by GC (GC 14A, Shimadzu) and GC–MS (320-MS Quadrupole GC/MS, Varian) of the *N*-heptafluorobutyryl amino acid

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, obtained from the GenBank/EMBL/DBJ database, of type strains of selected species of the genus *Microbacterium*. Multiple alignments, distance calculations (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were performed by using the software package MEGA version 4 (Tamura *et al.*, 2007). Bootstrap values >50 %, based on 1000 replications, are listed at branch points. Bar, 0.01 substitutions per nucleotide position.

isobutyl esters (MacKenzie, 1987; Groth *et al.*, 1996). Hydrolysates (treated with 4M HCl at 100 °C for 16 h) of the purified peptidoglycan of strain CC-VM-Y^T contained the amino acids ornithine (Orn), alanine (Ala), glycine (Gly), homoserine (Hsr) and glutamic acid (Glu) in an approximate molar ratio of 1.0:0.8:2.2:0.8:0.3. In addition, a substantial amount of *threo*-3-hydroxy glutamic acid (Hyg) was detected, which is characteristic of peptidoglycan type B2β (Schleifer & Kandler, 1972) (type B6 according to http://www.dsmz.de/microorganisms/main.php?content_id=35). The acyl type of the peptidoglycan was glycolyl. The menaquinones of strain CC-VM-Y^T were MK-13 (72 %), MK-12 (25 %) and MK-11 (3 %), similar to those found in other species of the genus *Microbacterium* (Li *et al.*, 2005; Young *et al.*, 2010). The polar lipids comprised phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), one unknown phospholipid (PL) and one unknown glycolipid (GL) (Supplementary Fig. S1, available in IJSEM Online). These major polar lipids have been detected in numerous species of the genus *Microbacterium* (Zlamala *et al.*, 2002; Rivas *et al.*, 2004; Kim *et al.*, 2005; Buczolits *et al.*, 2008; Park *et al.*, 2006; Young *et al.*, 2010) and are listed in the emended genus description of Takeuchi & Hatano (1998).

Fatty acid analysis was performed according to the method of Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain CC-VM-Y^T was very similar to those of closely related members of the genus *Microbacterium* (Supplementary Table S1), all of which contained anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0} as major fatty acids.

Results of comparative physiological characterization tests (Kämpfer *et al.*, 1991) on strains CC-VM-Y^T, *M. insulae* CCUG 54523^T, *M. hominis* DSM 12509^T and *M. hydrocarbonoxydans* DSM 16089^T, using identical test conditions, are given in Table 1 and in the species description. DNA–DNA hybridization experiments were performed with strain CC-VM-Y^T and *M. insulae* CCUG 54523^T, *M. hominis* DSM 12509^T and *M. hydrocarbonoxydans* DSM 16089^T according to the method of Ziemke *et al.* (1998). Strain CC-VM-Y^T showed relatedness values of 44.1 % with *M. insulae* CCUG 54523^T (39.9 % reciprocal analysis), 38.2 % with *M. hominis* DSM 12509^T (29.1 % reciprocal analysis) and 34.9 % with *M. hydrocarbonoxydans* DSM 16089^T (31.9 % reciprocal analysis).

Based on differences in peptidoglycan types, quinone systems, fatty acid and polar lipid profiles and observed phenotypes between the strains as well as on the results of the DNA–DNA pairing studies and 16S rRNA gene analyses, strain CC-VM-Y^T represents a novel species of the genus *Microbacterium*, for which the name *Microbacterium arthrosphaerae* sp. nov. is proposed.

Description of *Microbacterium arthrosphaerae* sp. nov.

Microbacterium arthrosphaerae (ar.thro.spha.e'rae. N.L. gen. n. *arthrosphaerae* of *Arthrosphaera*, the genus name

Table 1. Assimilation of substrates by strain CC-VM-Y^T and type strains of the most closely related species of the genus *Microbacterium*

Strains: 1, CC-VM-Y^T; 2, *M. insulae* CCUG 54523^T; 3, *M. hominis* DSM 12509^T; 4, *M. hydrocarbonoxydans* DSM 16089^T. All data was obtained from this study using methods according to Kämpfer *et al.* (1991). All strains hydrolysed aesculin, para-nitrophenyl (pNP)-alpha-D-glucopyranoside, pNP-beta-D-glucopyranoside pNP-beta-D-xylopyranoside, pNP-phenyl-phosphonate, L-alanine-para-nitroanilide (pNA) and L-proline-pNA. None of the strains hydrolysed pNP-beta-D-glucuronide, pNP-phosphorylcholine, 2-deoxythymidine-5'-pNP-phosphate and L-glutamate-gamma-3-carboxy-pNA. All strains utilized the following as sole sources of carbon: L-arabinose, p-arbutin, cellobiose, D-fructose, D-glucose, D-galactose, D-mannose, maltose, sucrose, salicin, trehalose, D-xylose, D-mannitol, maltitol, pyruvate, L-aspartate and L-proline. None of the strains were able to utilize adonitol, myo-inositol, *cis*-aconitate, *trans*-aconitate, adipate, azelate, mesaconate, suberate, phenylacetate, DL-3-hydroxybenzoate, phenylacetate and L-tryptophan. +, Positive; -, negative; (+), weakly positive.

Substrate	1	2	3	4
Assimilation of:				
N-Acetyl-D-galactosamine	(+)	–	–	+
N-Acetyl-D-glucosamine	–	–	+	+
Gluconate	+	–	–	+
α-Melibiose	+	(+)	–	–
L-Rhamnose	–	(+)	+	+
D-Ribose	+	–	–	+
D-Sorbitol	–	–	–	+
Putrescine	+	–	–	+
Acetate, propionate	(+)	–	+	(+)
4-Aminobutyrate	+	–	–	–
Citrate	+	–	+	+
Fumarate	–	+	+	+
Glutarate	+	–	–	+
DL-3-Hydroxybutyrate	(+)	+	+	–
Itaconate	–	–	–	+
DL-Lactate	(+)	–	+	+
L-Malate	–	+	+	–
Oxoglutarate	–	–	+	+
L-Alanine	+	–	+	+
β-Alanine	–	–	(+)	+
L-Histidine	+	–	–	+
L-Leucine	+	(+)	–	+
L-Ornithine	–	–	–	+
L-Phenylalanine	(+)	–	–	–
L-Serine	–	–	–	+
4-Hydroxybenzoate	–	–	–	+

of the pill millipede *Arthrosphaera magna*, from which the type strain was isolated).

Cells are short rods ~2–3 × 1–1.2 µm in size. Gram-reaction-positive. Oxidase- and catalase-positive. Display aerobic respiratory metabolism. Good growth occurs after 3 days of incubation on Nutrient agar, R2A agar and tryptone soy agar at 25–30 °C, forming yellow colonies.

The peptidoglycan is of the type B2 β , with the interpeptide bridge D-Glu (Hyg)–Gly–D-Orn. The major quinones are MK-13 and MK-12 with minor amounts of MK-11. The polar lipid profile comprises PG, DPG, an unknown PL and an unknown GL. The major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. Differential characteristics derived from physiological tests using identical conditions are given in Table 1.

The type strain, CC-VM-Y^T (=DSM 22421^T =CCM 7681^T), was isolated from the faeces of the pill millipede *Arthrosphaera magna* Attems from India.

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