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Micromonospora acroterricola sp. nov., a novel actinobacterium isolated from a high altitude Atacama Desert soil

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The GenBank accession numbers for the 16S rRNA gene and the whole genome sequence of the strain 5R2A7^T are MG725918 and QGKR00000000, respectively.

Keywords: *Micromonospora acroterricola*; polyphasic taxonomy; phylogeny, Atacama Desert soil.

Abstract

A *Micromonospora* strain, designated 5R2A7^T, isolated from a high altitude Atacama Desert soil was examined using a polyphasic approach. The isolate was found to have morphological, chemotaxonomic and cultural characteristics typical of members of the genus *Micromonospora.* The cell wall contains *meso*- and *hydroxy*-diaminopimelic acid, the major whole-cell sugars are glucose, ribose and xylose, the predominant menaquinones MK-10(H_4), MK-10(H_6), MK-10(H_8) and MK-9(H_6), the major polar lipids diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and an unknown glycolipid, and the predominant cellular fatty acids *iso*-$C_{16:0}$, *iso*-$C_{15:0}$ and 10-methyl $C_{17:0}$. The digital genomic DNA G+C content is 72.3 mol%. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain 5R2A7^T was closely related to *Micromonospora coriariae* DSM 44875^T (99.8%) and *Micromonospora cremea* CR30^T (99.7%), and was separated readily from the latter, its closest phylogenetic neighbour, based on *gyrB* and multilocus sequence data, by low average nucleotide identity (92.59%) and *in silico* DNA-DNA relatedness (51.7%) values calculated from draft genome assemblies, and by a range of chemotaxonomic and phenotypic properties. Consequently, the strain is considered to represent a novel species of *Micromonospora* for which the name *Micromonospora acroterricola* sp. nov. is proposed. The type strain is 5R2A7^T (=LMG 30755^T =CECT 9656^T).

The actinobacterial genus *Micromonospora* [1,2], the type genus of the family *Micromonosporaceae* [3,4] of the order *Micromonosporales* [5], currently encompasses 83 validly published species (www.bacterio.net/micromonospora.html), including the type species, *Micromonospora chalcea* [6,7]. Members of the genus typically form non-motile single spores on well developed, branched substrate mycelia, lack aerial hyphae, have whole-cell hydrolysates rich in *meso*- and/or *hydroxy*-diaminopimelic acid, arabinose and xylose; phosphatidylethanolamine as the diagnostic phospholipid in the cell membrane and produce complex mixtures of menaquinones and saturated and branched-chain fatty acids [7].

Comparative analyses of whole-genome sequences show that representative micromonosporae form a monophyletic group composed of four well supported lineages, two of which were recovered in their entirety in corresponding phylogenetic trees based on single gene sequences [8]. Carro and her colleagues [8] considered that *Micromonospora* strains should be given more prominence in the search for new classes of bioactive compounds as their genomes showed a much greater potential to synthesize specialized metabolites than previously realized. Micromonosporae are generally associated with aquatic and terrestrial habitats, notably soil, are being increasingly found in plant tissues, including nitrogen-fixing root nodules [7-9] and are a rich source of novel specialized (secondary) metabolites [10,11].

Culture-dependent and culture-independent surveys show that small numbers of novel, taxonomically diverse, filamentous actinobacteria, including micromonosporae, are a feature in Atacama Desert soils [12-14]. The presence of novel micromonosporal propagules in the Atacama Desert landscape provides a unique opportunity for bioprospecting, not least because it is now evident that genomes of micromonosporae contain taxon/taxa specific biosynthetic gene clusters thereby providing a way of prioritising gifted strains for genome mining and natural product discovery [13,14]. These developments also underline the merit of selecting

representatives of novel actinobacterial taxa in the search for new specialized metabolites [14,15].

The present study was designed to establish the taxonomic status of a putatively novel Atacama Desert isolate [13], strain 5R2A7^T, which had been shown to have a unique BOX-PCR fingerprint and which formed a well supported clade in a *Micromonospora* 16S rRNA gene tree together with the type strains of *Micromonospora coriariae* [16] and *Micromonospora cremea* [17]. The results of a comprehensive polyphasic taxomonic study show that strain 5R2A7^T represents a novel species within the genus *Micromonospora* for which we propose the name *Micromonospora acroterricola* sp. nov.

Isolate 5R2A7^T was recovered from a surface soil sample (2 cm) collected at 5041 meters on Cerro Chajnantor (23°00′49″S/67°45′31″W), adjacent to the Atacama Large Millimeter Array (ALMA) east of San Pedro de Atacama, Chile in November 2012 [18]. The strain was isolated on R2A agar [19] supplemented with cycloheximide and nalidixic acid (each at 50 µg ml^{-1}), using a standard dilution plate procedure [20] and incubation at 28 °C for 4 weeks. The isolate was transferred to R2A agar lacking antibiotics and found to produce typical micromonosporal-like colonies, that is, filamentous, orange coloured colonies that turned bluish-black upon spore formation. Strain 5R2A7^T and the type strains of *M. cremea* [17] and *M. coriariae* [16] and were maintained on modified Bennett's agar [23] and as hyphal fragments and spores in 20% v/v glycerol at – 80 °C for prolonged preservation. Biomass for the chemotaxonomic, 16S rRNA gene and whole-genome sequencing studies on isolate 5R2A7^T was prepared in shake flasks (180 revolutions per minute [rpm]) of yeast extract-malt extract broth (International *Streptomyces* Project medium 2 [ISP2]) [24] following incubation at 28 °C for 14 days and washed three times in sterile distilled water. Cells for the chemotaxonomic analyses were freeze-dried and those for the sequencing studies stored at room temperature.

Cell morphology, Gram staining and motility were observed on a phase-contrast microscope (Leica; CTR MIC) using 7-day-old cultures grown on ISP 2 agar. Strain 5R2A7^T was Gram-stain-positive, non-motile and showed extensively branched, non-fragmented substrate hyphae, but lacked aerial hyphae. Single, non-motile spores (0.7- 1.2 µm) were detected after 2 weeks of incubation (**Fig. S1**).

The cultural characteristics of the isolate were determined on modified Bennett's [22], Gauze's No. 1 [25] and SA1 agar [26] plates, as well as on ISP2 and oatmeal agar (ISP3) [24] following incubation at 28 °C for 14 days. The strain was also examined for its ability to grow in the presence of various concentrations of sodium chloride (1, 2, 5, 7 and 9% w/v) and over a range of pH (4.0-9.0 at one unit intervals) and temperature regimes (4, 10, 20, 28 37 and 40 °C) using GYM *Streptomyces* agar (DSMZ medium 65) [27] as the basal medium; pH values were established using phosphate buffers, as described previously [16]. The temperature tests were recorded after 7 (40 °C), 14 (20, 28 and 37 °C) and 21 (4, 10°C) days and the remaining ones following growth at 28 °C for 14 days. The isolate grew well on all media producing characteristic colonies; aerial hyphae were not formed. Growth was observed at 20-37 °C, optimally at 28 °C, but not at 4, 10 or 40 °C; from pH 6-9, optimally at pH 8, but not at pH 4-5 or 10, and in the presence of maximum 1% w/v sodium chloride, optimally without NaCl.

Strain 5R2A7^T was examined for chemotaxonomic markers considered to be characteristic of strains assigned to the genus *Micromonospora* [7]. Standard procedures were used to detect isomers of diaminopimelic acid (A_2pm) [28], menaquinones [29], polar lipids [30] and whole cell sugar composition [31], using appropriate controls. The acyl type of the cell-wall muramic acid was determined according to method of Uchida *et al*. [32]. Cellular fatty acids were extracted, methylated, determined using gas chromatography (Agilent Technologies, mod. 7890A GC System), and analyzed using the protocol of the Sherlock

Microbial Identification (MIDI) system, version 5 [33]; the resultant peaks were named using the RTSBA6 database.

The chemotaxonomic properties of the isolate were consistent with its assignment to the genus *Micromonospora* [7,8]. The peptidoglycan contains a mixture of *meso*- and *hydroxy*-A_2pm, the diagnostic whole-organism sugars are ribose and xylose, the cell wall muramic type is glycolyl, and the predominant menaquinones MK-10(H_4), MK-10(H_6), MK-10(H_8) and MK-9(H_6) in the ratio of 19, 26, 28, and 10%, respectively, and the major polar lipids diphosphatidylglycerol, phosphatidylethanolamine (diagnostic phospholipid), phosphatidylinositol and an unknown glycolipid (**Fig. S2**). The isolate contained major proportions of *iso*-$C_{15:0}$ (20% of total), *iso*-$C_{16:0}$ (19.3%), $C_{17:0}$ (8.4%) and 10-methyl $C_{17:0}$ (10.0%), lesser proportions of *iso*-$C_{16:1}$ (2.2%), $C_{16:0}$ (2.0%), *iso*-$C_{17:0}$ (4.0%), *anteiso*-$C_{17:0}$ (7.7%), *iso*-$C_{17:1}$ ω9c (2.9%), $C_{17:1}$ ω8c (4.2%), $C_{18:1}$ ω9c (2.3%), $C_{18:0}$ (1.7%) and trace amounts (<1.0) of *iso*-$C_{13:0}$, $C_{13:0}$, $C_{14:0}$, *iso*-G $C_{15:1}$, $C_{15:1}$ ω8c, *anteiso*-$C_{16:0}$, *anteiso*-$C_{17:1}$ ω9c, *iso*-$C_{18:0}$, $C_{19:0}$, $C_{18:1}$ 2OH and $C_{20:1}$ ω9c.

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene of the isolate and direct sequencing of the purified PCR product were performed, as described by Golinska *et al*. [34,35], resulting in an almost complete 16S rRNA gene sequence (1413 nucleotides [nt]) (GenBank accession number: MG725918). Corresponding sequences of the type strains of closely related *Micromonospora* species were found using the EzBioCloud server [36] then aligned using Clustal W [37]. Phylogenetic trees were constructed using the neighbour-joining [38] and maximum-likelihood [39] algorithms drawn from the MEGA7 program [40]; the resultant trees were evaluated in bootstrap analyses based on 1000 replicates [41]. Evolutionary distances were calculated using the two-parameter model of Kimura [42]. In addition, a multilocus sequence analysis (MLSA) based on 16S rRNA, *atpD*, *gyrB*, *recA* and *rpoB* gene sequences was carried out using established procedures [43] and a

MLSA tree generated from the 10320 nucleotides using the neighbour-joining and maximum-likelihood algorithms. Similarly, a *gyrB* tree based on 1967 nucleotides was generated following the procedure described by Garcia et al. [44].

It can be seen from the phylogenetic trees (**Fig. 1 and Fig. S3**) that isolate 5R2A7^T forms a well supported 16S rRNA clade together with *M. coriariae* DSM 44875^T and *M. cremea* DSM 45599^T; the type strains of *M. chersina* [45] and *M. endolithica* [46] are loosely associated with this taxon. The isolate shares 16S rRNA gene sequence similarities with the *M. coriariae* and *M. cremea* strains of 99.8 and 99.7% respectively, values that correspond to 3 and 5 nt differences at 1407 locations; the corresponding sequence similarities with the *M. chersina* and *M. endolithica* strains were 99.3 and 99.2%. Strain 5R2A7^T also shared quite high 16S rRNA gene sequence similarities with the type strains of *M. inositola* [47] and *M. terminaliae* [48], namely 99.0 and 99.1%, respectively; corresponding similarity scores with the remaining *Micromonospora* type strains were below the 98.5% threshold used to distinguish between closely related prokaryotic species [49].

The results of the present study provide further evidence that micromonosporal phylogenies generated from *gyrB* and concatenated sequences of housekeeping genes show greater resolution between constituent strains than corresponding trees derived from analyses of 16S rRNA gene sequences [13,17,43,50]. It can be seen from the *gyrB* tree that isolate 5R2A7^T forms a well supported clade together with *M. cremea* DSM 45599^T, while the type strains of *M. chersina, M. coriariae, M. endolithica* and *M. inositola* form distinct branches in other parts of the tree (**Fig. 2**). Even better resolution was found between the strains in the MLSA tree based on the four housekeeping genes (**Fig. 3**). It is evident from this tree that isolate 5R2A7^T forms a well supported clade together with the type strains of *M. cremea* and *M. coriariae,* appearing also related to the type strains of *M. chokoriensis* [21], *M. lupini* [22], *M. saelicesensis* [22] and *M. zamorensis* [17]; all of these strains formed well delineated clades in

the MLSA and phylogenomic trees generated by Carro et al [8]; with the exception of *Micromonospora chokoriensis* DSM 45160^T all of the type strains of these species were isolated from ecto- and endo-rhizospheres. It is clear from both the *gyrB* and MLSA trees that isolate 5R2A7^T is most closely related to *M. cremea* DSM 45599^T.

A single colony of strain 5R2A7^T was used to inoculate 50 ml of GYM broth which was then incubated in a shake flask (180 rpm) for 72 hours at 28 °C. Genomic DNA was extracted from spun-down biomass and sequenced at MicrobesNG on a MiSeq instrument (Illumina). The various reads were assembled into contigs using Spades 3.6.2 software [51] and contigs under 500bp discarded. The draft assembly of this publically available genome (GenBank QGKR00000000) is composed of 369 contigs giving a total size of 6510137 bp with a digital DNA G+C content of 72.3 mol%. Digital DNA-DNA hybridization (dDDH) similarities were determined between the genome of strain 5R2A7^T and the genomes of the type strains of its closest phylogenetic neighbours (**Fig.3**) using the GGDC server [52]. Similarly, the average nucleotide identity (ANI) between the genome of the isolate and the genomes of the type strains of its nearest phylogenetic neighbours were calculated according to Rodriguez and Konstantinidis [53]. The resultant *in silico* DNA:DNA pairing values between isolate 5R2A7^T and the type strains of *M. cremea, M. coriariae, M. chokoriensis*, *M. lupini, M. saelicesensis* and *M. zamorensis* were 51.7, 49.6, 37.8, 40.9, 43.3 and 42.1% respectively, values well below the 70% cut-off point widely used for the delineation of prokaryotic species [54]. The corresponding ANI similarities between strain 5R2A7^T and the six species mentioned above were 92.59, 92.11, 88.52, 89.53, 89.36 and 89.48%, values considerably below the 95-96% threshold used to distinguish between closely related species [49,55].

The genome of strain 5R2A7^T was examined for gene clusters encoding for natural products using anti-SMASH 4.0 [56] while *nif* genes were sought using the SEED viewer [57] following RAST annotation of the genome [58,59]. As expected the organism did not contain

nif genes, a result in line with previous studies on micromonosporae [8]. In contrast the genome of strain 5R2A7^{T} contained 12 biosynthetic gene clusters (BGC's) including ones associated with the production of desferrioxamine B, sioxanthin, sap B, as well as ones encoding for bacteriocins, lantipeptides, nonribosomal peptide synthetase, polyketide synthases, siderophores and terpenes. The number of bioclusters found in strain 5R2A7^{T} is within the range (9-16) found in other members of the group IVa [8] but is well below the average number associated with *Micromonospora* strains (20). It is also interesting that all but one of the 12 BGC's are associated with compounds detected in closely related *Micromonospora* strains, the exception encodes for a product related to fengyacin, antifungal lipopeptide originally described in *Bacillus subtilis* [60]. This compound has also been detected in three *Micromonospora* strains isolated from Atacama Desert soil, *Micromonospora ureilytica* LB 19, *Micromonospora arida* LB 32^{T} and *Micromonospora inaquosa* LB 39^{T} [61] but not in micromonosporae isolated from other habitats [8].

Strain 5R2A7^{T} was examined for a range of standard biochemical, degradative and physiological tests [62] which had been used previously to acquire data on its nearest phylogenetic neighbours, namely and *M. cremea* DSM 45599^{T} [17] and *M. coriariae* DSM 44875^{T} [16]. The enzyme properties of strain 5R2A7^{T} were determined using API ZYM kits, according to the manufacturer's instructions; the latter had previously been followed to acquire corresponding data on the two strains mentioned above. A standard inoculum corresponding to 5 on the McFarland scale [63] was used to inoculate the tests carried out on strain 5R2A7^{T}. In addition, the ability of strain 5R2A7^{T} and the type strains of M. cremea and *M. coriariae M.* to oxidise diverse carbon and nitrogen sources and to show resistance to inhibitory compounds were determined using GEN III microplates in an Omnilog device (BIOLOG Inc., Haywood, USA) using the opm package R version 1.06 [64,65], these tests were carried out in duplicate.

The isolate can be distinguished from the type strains of all its closest phylogenetic neighbours using a combination of chemotaxonomic and other phenotypic properties (Table 1). In particular, it can be separated from *M. cremea* DSM 45599^{T}, its overall closest phylogenetic relative, as unlike the latter, it gave positive results for α- and β-galactosidase and α-mannosidase. The corresponding BIOLOG data based on duplicated data showed that the isolate, but not the *M. cremea* strain, grew at pH 6.0 and metabolized D-fructose, D-galactose, β-gentiobiose, *myo*-inositol, D-mannose, glycyl-L-proline, D-sucrose, D-trehalose, D-turanose, bromo-succinic acid and α-*keto*-glutaric acid. In contrast, only *M. cremea* DSM 45599^{T} utilized N-acetyl-D-galactosamine, D-glucose, glycerol, D-mannitol, inosine, butyric acid, N-acetyl-neuraminic acid, *p-hydroxy*-phenylacetic acid, L-pyroglutamic acid and quinic acid, and was not inhibited by potassium tellurite, tetrazolium blue or tetrazolium violet. It is also clear from Table 1 that a broad range of phenotypic features can be used to distinguish strain 5R2A7^{T} from the type strain of *M. cremea* and *M. coriariae.*

In short, strain 5R2A7^{T} can be distinguished from all members of *Micromonospora* phylogenomic group IVa [8], based on *gyrB* and MLSA gene sequences, low ANI and dDDH scores. A corresponding wealth of taxonomic data separate strain 5R2A7^{T} from *M. cremea* DSM 45599^{T}, its closest phylogenetic neighbour. It is clear from these datasets that strain 5R2A7^{T} represents a new centre of taxonomic variation within the genus *Micromonospora*, the name chosen for thus species is *Micromonospora acroterricola* sp. nov. The Digital Protologue database Taxonumber for the strain is CA00035.

Description of *Micromonospora acroterricola* sp. nov.

Micromonospora acroterricola (a.cro.ter.ri'co.la. Gr. adj. *akros*, high, at the top end; L. n. *terra*, soil; L. suff.-*cola* (from L. n. *incola*, dweller, inhabitant; N.L. n. *acroterricola*, an inhabitant of high lands).

Aerobic, Gram-stain-positive, chemoorganotrophic actinobacterium which forms non-motile single spores (0.7 – 1.2 µm) on well developed, extensively branched substrate hyphae (diameter 0.5 – 1.0 µm), but does not produce aerial hyphae. Colonies are orange on ISP2 agar eventually turning bluish-black on sporulation. Growth occurs between 20-37 ^{o}C, optimally at 28 ^{o}C, from pH 6 to 9, optimally around pH 8 and in presence up to 1% w/v NaCl. Degrades casein, gelatin and Tween 40, but not pectin, and is catalase and oxidase positive, produces α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α- and β-galactosidase, β-gentiobiose, N-acetyl-β-glucosaminidase, α- and β-glucosidase, β-glucuronidase, leucine arylamidase, lipase (C14), α-mannosidase acid and alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but not α-fucosidase. Oxidizes L-alanine, L-arginine, L-aspartic acid, L-glutamic acid and glycyl-L-proline, but not D-aspartic acid, L-histidine, pyro-glutamic acid or D- and L-serine (amino acids), and D-cellobiose, D-fructose, D- and L-fucose, L-galactonic acid-γ-lactone, D-galactose, N-acetyl-D-glucosamine, β-methyl-D-glucoside, D-glucuronamide, *myo*-inositol, α-D-lactose, D-maltose, N-acetyl-β-D-mannosamine, D-mannose, D-melibiose, D-fructose-6-phosphate, D-glucose-6-phosphate, D-raffinose, L-rhamnose, D-salicin, D-sorbitol, sucrose, stachyose, D-trehalose, D-turanose, but not D-arabitol, dextrin, N-acetyl-D-galactosamine, D-glucose, 3-*O*-methyl-D-glucose, glycerol or D-mannitol (sugars) and acetic acid, acetoacetic acid, γ-amino-*n*-butyric acid, α- and β-*hydroxy*-D,L-butyric acid, D-galacturonic acid, D-glucuronic acid and D-gluconic acid, α-keto-glutaric acid, D- and L-lactic acid, D- and L-malic acid, propionic acid, methyl-pyruvate and bromo-succinic acid, but not butyric acid, α-*keto*-butyric acid, citric acid, mucic acid, N-acetyl-neuraminic acid, *p-hydroxy*-phenylacetic acid, quinic acid and D-saccharic acid (organic acids). Sensitive to fusidic acid, guanidine hydrochloride, lincomycin, minocycline, troleandomycin and vancomycin, but resistant to aztreonam, nalidixic acid and rifamicin SV. It is inhibited by lithium chloride, niaproof,

sodium bromate, sodium formate, sodium lactate (1% w/v), potassium tellurite, tetrazolium blue and tetrazolium violet. Additional phenotypic data are given in the text and Table 1. The cell wall contains *meso*- and *hydroxy*-A_2pm, the whole-cell sugars are glucose, ribose and xylose, the major fatty acids *iso*-$C_{16:0}$, *iso*-$C_{15:0}$ and 10-methyl $C_{17:0}$, the predominant menaquinones MK-10(H_4), MK-10(H_6), MK-10(H_8) and MK9(H_6) and phosphatidylethanolamine is the diagnostic phospholipid. The dDNA G+C content of the type strain is 72.3 mol% and its genome size around 6.5 Mbp.

The type strain, 5R2A7^T (=LMG 30755^T, =CECT 9656^T) was isolated from a surface sample of a high altitude soil collected from Cerro Chajnantor, near San Pedro de Atacama, Chile. The GenBank accession numbers for the 16S rRNA gene and the whole genome sequence of the strain 5R2A7^T are MG725918 and QGKR00000000, respectively.

To date, two *Micromonospora* species, *Micromonospora arida* and *Micromonospora inaquosa*, have been isolated from Atacama Desert soils [61]. However, it is unlikely that *M. acroterricola* will be the last such species as putatively novel micromonosporae isolated from this extreme biome have been highlighted based on comparative 16S rRNA gene sequence data [13] and in metagenomics surveys [18]. These results square with those from culture-independent studies where small numbers of micromonosporae were found to be a feature of several Atacama Desert habitats [12,66].

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical statement

The authors have not carried out any studies involving human participants or animals.

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Table 1. Phenotypic properties that distinguish strain 5R2A7[T] from its closest phylogenetic relatives. Strains: 1, 5R2A7[T]; 2, *M. coriariae* DSM 44875[T] and 3, *M. cremea* DSM 45599[T]. +, positive; -, negative. All data are from this study.

Characteristic	**1**	**2**	**3**
Colour of substrate mycelium on ISP2	intense orange	intense orange	cream to orange
Temperature range	20-37	12-37	10-37
pH range	6-9	6-9	7-8
Maximum NaCl tolerance (%, w/v)	1	8	1
API ZYM tests			
α-Chymotrypsin	+	-	+
α -Galactosidase	+	+	-
β -Galactosidase	+	+	-
α -Mannosidase	+	-	-
Naphthol-AS-BI-phosphohydrolase	+	-	+
GENIII BIOLOG microplate tests			
(a) Oxidation of amino acids			
Glycyl-L-proline	+	-	-
L-Pyroglutamic acid	-	+	+
(b) Oxidation of nucleoside			
Inosine	-	-	+
(c) Oxidation of sugars			
Dextrin	-	+	-
D-Fructose	+	+	-
D-Fucose	+	-	+
L-Fucose	+	-	+
D-Galactose	+	+	-
N-acetyl-β-D-Galactosamine	-	-	+
β-Gentiobiose	+	+	-
D-Glucose	-	+	+
Glucuronamide	+	-	+
Glycerol	-	+	+
myo-Inositol	+	+	-
α-D-Lactose	+	-	+
D-Mannitol	-	+	+
N-acetyl-D-Mannosamine	+	-	+
D-Mannose	+	+	-
D-Melibiose	+	-	+
D-fructose-6-phosphate	+	-	+
D-Fructose-6-Phosphate	+	-	+
L-Rhamnose	+	-	+
D-Sucrose	+	+	-
D-Trehalose	+	+	-

Turanose	+	+	-
(c) Oxidation of organic acids			
Acetoacetic acid	+	-	+
Butyric acid	-	+	+
α-*hydroxy*-D,L-Butyric acid	+	-	+
β-*hydroxy*-D,L-Butyric acid	+	-	+
Citric acid	-	+	-
α-keto-Glutaric acid	+	-	-
D-Lactic acid methyl ester	+	-	+
N-acetyl-Neuraminic acid	-	-	+
p-hydroxy-Phenylacetic acid	-	-	+
Quinic acid	-	-	+
D-saccharic acid	-	+	-
Bromo-Succinic acid	+	-	-
Resistance of inhibitory compounds:			
Lithium chloride	-	+	-
Potassium tellurite	-	+	+
Sodium bromate	-	+	-
Sodium chloride (1%)	-	+	+
Sodium chloride (4%)	-	+	-
Sodium chloride (8%)	-	+	-
Sodium lactate (1%)	-	+	-
Tetrazolium blue	-	-	+
Tetrazolium violet	-	+	+
Rifamicin SV	+	-	+
Growth in presence of NaCl:			
1% w/v	-	+	+
4% w/v	-	+	-
8% w/v	-	+	-
Growth at pH 6	+	+	-

All strains oxidize L-alanine, L-aspartic acid, L-glutamic acid, but not D-aspartic acid, L-histidine or D-serine (amino acids), D-cellobiose, N-acetyl-D-glucosamine, β-methyl-D-glucoside, D-maltose, D-raffinose, salicin, stachyose, but not D-arabitol (sugars), acetic acid, D-gluconic acid, malic acid, propionic acid and methyl-pyruvate, but not α-keto-butyric acid, fusidic acid or mucic acid (oranic acids). All strains were resistant to aztreonam and nalidixic acid, but sensitive guanidine hydrochloride, lincomycin, minocycline, troleandomycin or vancomycin, and do not grow in pH 5 and presence of niaproof.

Legends for Figures

Fig. 1. Neighbour-joining phylogenetic tree based on almost complete 16S rRNA gene sequences showing relationships between isolate 5R2A7^{T} and closely related *Micromonospora* type strains. The numbers at the nodes are bootstrap support values when > 50%. Asterisks indicate branches of the tree that were also recovered in the maximum-likelihood tree. *Catellatospora citrea* DSM 44097^{T} was used as the outgroup. Bar, 0.005 substitutions per nucleotide position.

Fig. 2. Neighbour-joining phylogenetic tree based on almost complete *gyr*B gene sequences showing relationships between isolate 5R2A7^{T} and *Micromonospora* type strains. The numbers at the nodes are bootstrap support values when > 50%. Asterisks indicate branches of the tree that were also recovered in the maximum-likelihood tree. *Catellatospora citrea* DSM 44097^{T} was used as the outgroup. Bar, 0.02 substitutions per nucleotide position.

Fig. 3. Neighbour-joining phylogenetic tree based on multilocus sequence aligment of 16rRNA, *gyr*B, *rpo*B, *atp*D, and *rec*A gene sequences showing relationships between isolate 5R2A7^{T} and *Micromonospora* type strains. The numbers at the nodes are bootstrap support values when > 50%. Asterisks indicate branches of the tree that were also recovered in the maximum-likelihood tree. *Catellatospora citrea* DSM 44097^{T} was used as the outgroup. Bar, 0.005 substitutions per nucleotide position.

Fig. S1. Phase contrast image of strain 5R2A7^{T} grown on M65 agar at 28 ^{o}C for 3 weeks showing spores at the top of hyphae. Stained with methylene blue. Bar 5 µm.

Fig. S2. Two dimensional thin layer chromatograph of polar lipids of strain 5R2A7^{T} stained with molybdenum blue (Sigma). Chloroform:methanol:water (32.5:12.5:2.0 v/v) was used in the first direction and chloroform:glacial acetic acid:methanol:water (40:7.5:6:2

v/v) in the second direction. DPG, diphosphatidylglycerol; GL, glycolipid; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

Fig. S3. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequence comparisons showing the position of strain 5R2A7^T relative to the type strains of *Micromonospora* species. The numbers at the nodes indicate bootstrap values > 50%. Bar, 0.005 substitutions per nucleotide position.