***Micromonospora orduensis* sp. nov., isolated from deep marine sediment**

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Abstract

A novel actinobacterial strain, designated S2509^T, was isolated from marine sediment collected by a dredge at a depth of 45 m along Melet River offshore of the southern Black Sea coast, Ordu, Turkey. The cell wall peptidoglycan of strain was found to contain *meso*-diaminopimelic acid and 3-OH-diaminopimelic acid. The whole cell sugars detected were arabinose, glucose, rhamnose, ribose and xylose. The diagnostic phospholipids of strain S2509^T were found to be diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, a glycolipid and two unidentified phospholipids. The predominant menaquinones were identified as MK-9(H₈), MK-9(H₆), MK-10(H₈), MK-9(H₄), MK-10(H₄) and MK-10(H₆). The major cellular fatty acids were found to be *iso*-C_{16:0}, *iso*-C_{15:0} and 10-methyl C_{17:0}. The taxonomic position of the strain was established using a polyphasic approach, showing that S2509^T strain belongs to the genus *Micromonospora*. Phylogenetic analysis based on the 16S rRNA gene sequence of strain S2509^T showed that it is closely related to the type strain of *Micromonospora chokoriensis* DSM 45160^T (99.37 % sequence similarity), and phylogenetically clustered with *Micromonospora inaquosa* LB39^T (99.37 %), *Micromonospora lupini* Lupac 14N^T (99.16 %), *Micromonospora violae* NEAU-zh8^T (99.23 %) and *Micromonospora taraxaci* NEAU-P5^T (99.03 %). The phylogenetic analysis based on the *gyrB* gene sequence of strain S2509^T confirmed its close relationship with *M. chokoriensis* JCM 13247^T (96.5 % sequence similarity). Whole genome sequences confirmed by digital DNA-DNA hybridization analysis that the strain S2509^T represents a novel species in the genus *Micromonospora*, for which the name *Micromonospora orduensis* sp. nov. is proposed. The type strain is S2509^T (=DSM 45926^T = KCTC 29201^T).

Keywords: *Micromonospora*, polyphasic taxonomy, marine sediment, 16S rRNA gene, *gyrB* gene

Introduction

The genus *Micromonospora* was described by Ørskov in 1923 (Kawamoto 1989), and at the time of writing, the genus comprised 84 species with validly published names (www.bacterio.net/micromonospora.html). Micromonosporae are frequent inhabitants of aquatic habitats worldwide where they participate in the decomposition of cellulose, chitin and lignin (Cross et al. 1989; Gullo et al. 2006). *Micromonospora* strains have been isolated from water samples from streams, rivers and lakes, from lake mud, river sediments, beach sands, littoral sediments and deep marine sediments (Cross et al. 1989; Goodfellow and Williams 1983; Jensen et al. 2005). Micromonosporae have been found to be the dominant actinomycetes group in a range of aquatic environments, particularly in the deeper mud layers, as well as in deep sea sediments (Rusnak et al. 2001; Mincer et al. 2002). In recent years many *Micromonospora* species have been isolated from sea sediment, as exemplified by *Micromonospora fluostatini* (Phongsopitanun et al. 2015), *Micromonospora yasonensis* (Veyisoglu et al. 2016), *Micromonospora profundus* (Veyisoglu et al. 2016) and *Micromonospora globispora* (Carro et al. 2019). In the present study, we describe a novel species isolated from a Black Sea deep sediment. The resultant data showed that isolate S2509^T represents a novel *Micromonospora* species for which the name *Micromonospora orduensis* sp. nov. is proposed.

Materials and methods

Isolation, maintenance and cultural conditions of strains

Strain S2509^T was isolated from marine sediments collected by a dredge at a depth of 45 m, offshore of the Melet river, Turkey (GPS coordinates for sampling site are 41°00.353' N and 37°57.489' E). The sediment samples were stored at -20 °C until examined using a standard dilution plate method. The diluted sediment suspensions were inoculated on SM3

medium (Gauze's medium 2) [20 g casamino acids, 20 g soluble starch, 4 g yeast extract, 15 g agar, pH 7.2-7.4 per litre distilled water] (Tan et al. 2006) supplemented with filter sterilised cycloheximide (50 $\mu\text{g ml}^{-1}$), nalidixic acid (10 $\mu\text{g ml}^{-1}$), novobiocin (10 $\mu\text{g ml}^{-1}$) and nystatin (50 $\mu\text{g ml}^{-1}$), incubated at 30 °C for 30 days. The strain was isolated as a pure culture and maintained on yeast extract-malt extract agar [International Streptomyces Project medium 2 [ISP 2]; (Shirling and Gottlieb 1966)] slopes at room temperature and preserved as suspensions of mycelial fragments and spores in glycerol (25 %, v/v) at -80 °C. *Micromonospora chokoriensis* DSM 45160T (Ara and Kudo 2007) was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures) and cultured under comparable conditions as a reference strain.

Morphological, cultural, physiological and biochemical characteristics

The micromorphological properties of strains were determined by examining gold coated dehydrated specimens of 43-days cultures N-Z-Amine (DSMZ Medium 554) using a JEOL JSM 6060 instrument. Cultural characteristics were determined after growth for 14 days at 30 °C as described by Shirling and Gottlieb (1966) on ISP 2, ISP 3, ISP 4, ISP 5, ISP 6, ISP 7 and modified Bennett's agar (Jones 1949), Czapek's, nutrient and tryptic soy agar (Difco). ISCC-NBS Colour Name Charts Standard Samples No 2106 (Kelly 1964) was used to determine the colour of colonies and soluble pigments. Tolerance of various temperatures (4, 10, 20, 28, 37, 40, 45, 50 and 55 °C), NaCl concentrations (1-10 NaCl (w/v) at intervals of 1.0 NaCl unit) and pH (4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11 and 12) were determined on ISP 2 at 30 °C for 14 days; $\text{KH}_2\text{PO}_4/\text{HCl}$, $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ and $\text{K}_2\text{HPO}_4/\text{NaOH}$ buffer systems were used to maintain the pH values of the media. Utilisation of sole carbon sources was tested using ISP 9 (pH 9) (Shirling and Gottlieb 1966) and supplemented with a final concentration of 1 % (w/v) of the corresponding carbon source. Utilisation of amino acids as nitrogen sources was tested as described by

Williams et al. (1983) supplemented with a final concentration of 0.1 % (w/v) of the nitrogen sources. The degradation tests were examined using the method described by Williams et al. (1983). Hydrolysis of arbutin, allantoin, aesculin, urea and nitrate were determined as described by Collins et al. (2004) and that of Tweens (40 and 80) according to the method of Nash and Krent (1991).

Chemotaxonomic characteristics

Biomass for chemical studies was obtained by growing strain S2509T in GYM medium (DSMZ Medium 65) in shake flasks (200 r.p.m.) at 30 °C for 14 days. Cells were harvested by centrifugation, washed twice with sterile distilled water and freeze-dried (Minnikin et al. 1984). Whole cell sugar composition was prepared according to Lechevalier and Lechevalier (1970) and analysed by thin layer chromatography (Staneck and Roberts 1974). Standard thin layer chromatographic procedures were used to establish the chemotaxonomic profiles of the strains, including the determination of the isomers of diaminopimelic acid (A2pm) (Staneck and Roberts 1974), predominant isoprenologues (Collins 1985; Kroppenstedt 1985), diagnostic sugars and polar lipids, using the procedure of Minnikin et al. (1984). Cellular fatty acids were extracted, methylated and separated by gas chromatography using an Agilent Technologies 6890 N instrument, fitted with an autosampler and a 6783 injector, according to the standard protocol of the Sherlock Microbial identification (MIDI) system (Sasser 1990; Kämpfer and Kroppenstedt 1996); the fatty acid methyl ester peaks were quantified using the TSBA 5.0 database.

DNA preparation, amplification and determination of 16S rRNA and *gyrB* gene sequences
Genomic DNA extraction, PCR-mediated amplification and 16S rRNA gene sequencing were performed as described by Chun and Goodfellow (1995), using an ABI PRISM 3730 XL automatic sequencer. The resultant 16S rRNA gene sequences were aligned with

corresponding sequences of representative type strains of the genus *Micromonospora* (retrieved from the EzBioCloud server; Yoon et al. 2017) by using CLUSTAL W in MEGA version 7.0 software (Tamura et al. 2013). PCR amplification and *gyrB* gene sequencing were carried out on strain S2509T using primers GYF1 and GYR3B, as described by Garcia et al. (2010). Phylogenetic analyses were carried out using the neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) algorithms from the MEGA version 7.0 software. Evolutionary distances were calculated using the Kimura two-parameter (Kimura 1980) and topologies of the resultant trees evaluated by bootstrap analyses (Felsenstein 1985) based on 1000 resamplings. *Catellatospora citrea* DSM 44097T (16S rRNA gene sequence GenBank accession number X93197) was used as the outgroup.

Whole genome sequencing

The genome of S2509T was sequenced using the Illumina HiSeq 2500 platform (250 p paired-end reads) at Microbes NG and analysed with a standard pipeline; to determine the closest available genome and mapping the reads against it Kraken and BWA mem software were used, respectively. SPAdes was used to generate a de novo assembly of the generated reads. VarScan was used for variant calling and MUMmer alignment to reorder and reorient the assembly relative to the reference genome. Prokka was used to perform an automated annotation. Genome base content was used to calculate GC content of the strain. Previously generated genomes were used for comparison (Carro et al., 2018). A whole genome phylogeny was generated using the TYGS server (<http://tygs.dsmz.de>) (Meier-Kolthoff and Göker, 2019). The whole genome sequence generated was used to determine OGRI values with respect to closely related type strains, including the digital DNA–DNA hybridization (dDDH) value calculated using formula 2 of the GGDC web server available

at <http://ggdc.dsmz.de/ggdc.php#> and OrthoANI value using OAT version 0.93.1 (Lee et al. 2016).

Results and discussion

Morphological observation of a 43-day-old culture of strain S2509T grown on N-Z-Amine agar revealed it had the typical characteristics of members of the genus *Micromonospora*. Strain S2509T was found to be non-motile and to bear single elliptical to oval spores (0.4 x 0.6 μ m) with smooth surfaces on branched substrate hyphae without fragmentation (Fig. S1). Good growth was observed on ISP2, ISP3, modified Bennett's and tryptic soy agar; moderate growth was observed on ISP6 and nutrient agar; poor growth was observed on ISP4, ISP5, ISP7 and Czapek's agar. The colour of the colonies varied, from light orange to dark brown; dark brown on ISP 3, dark orange on ISP 2 and ISP7, light orange on ISP 4, ISP 5, ISP 6, modified Bennett's, nutrient, Czapek's and tryptic soy agars. No diffusible pigments were observed on any of the tested media, including ISP 6 and ISP 7 agars (Table S1).

Growth of strain S2509T was found to occur in the pH range 5-11 and 0-3 % NaCl (w/v), with optimum growth at pH 7.0 and 0 % NaCl (w/v). Strain S2509T was found to grow at 28-40 °C, optimally $\approx$ 30 °C. Detailed physiological properties are shown in the species description and Table 1.

Cells of strain S2509T were observed to contain meso-diaminopimelic acid and 3-OH-diaminopimelic acid. Whole cell hydrolysates were found to contain arabinose, glucose, rhamnose, ribose and xylose. The phospholipid profile was found to contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, a glycolipid and two unidentified phospholipids (Fig. S2). The predominant menaquinones detected were

170 MK-9(H8) (18.7 %), MK-9(H6) (18.7 %), MK-10(H8) (17.6 %), MK-9(H4) (15.9 %), MK-

10(H4) (14.6 %) and MK-10(H6) (14.4 %). The cellular fatty acid profile was determined to be composed of iso-C16:0 (28.1 %), iso-C15:0 (17.0 %), 10-methyl C17:0 (12.0 %), anteiso-C17:0 (7.9 %), C17:1w8c (5.9 %) and C17:0 (5.5 %) (0.4 %) (Table 2).

The almost complete 16S rRNA gene sequence of strain S2509T (1475 bp) was determined and deposited as KF494805 in the GenBank/EMBL/DDBJ databases. Based on the EzTaxon-e analysis, strain S2509T was affiliated to the genus *Micromonospora* positioned in an independent branch and clustered with *M. chokoriensis* DSM 45160T (99.37 %), *Micromonospora inaquosa* LB39T (99.37 %), *Micromonospora violae* NEAU-zh8T (99.23 %), *Micromonospora lupini* Lupac14NT (99.16 %) and *Micromonospora taraxaci* NEAU-P5T (99.03 %) (Fig. 1 and Fig. S3), a relationship that was supported by the corresponding maximum-likelihood and maximum-parsimony analyses (Fig. S4 and Fig. S5), although with low bootstrap support.

The partial *gyrB* gene sequence obtained for strain S2509T (1131 nt) has been deposited in the GenBank/EMBL/DDBJ databases as KF818378. The *gyrB* sequence analysis showed that the strain S2509T forms an independent branch clustered with the type strains of *M. chokoriensis* DSM 45160T (96.5 %) and *M. taraxaci* NEAU-P5T (96.3 %), a relationship that was supported by the corresponding maximum-likelihood and maximum-parsimony analyses and by a high bootstrap value (Fig. 2 and Fig. S6). The phylogenetic analyses based on 16S rRNA gene and *gyrB* gene sequences confirmed that strain S2509T clustered together with the type strain of *M. chokoriensis*.

The draft genome sequence of the strain S2509T presents a genome size of 6898708 bp, comprised of 321 contigs, with 6,535 protein coding genes, 57 RNA (5 rRNA) and 256 pseudogenes. The N50 value is 40,544 and the DNA G+C content of strain S2509T was found to be 71.7 mol %. Whole genome phylogeny show S2509T forms independent branch and confirmed that *M. chokoriensis* DSM45160T and *M. taraxaci* DSM45885T are closely

related type strains, and that the strain is also closely related to *M. lupini* Lupac 08 (Figure 3). In silico DNA:DNA pairing between the genome of strain S2509T and the type strains of *M. chokoriensis*, *M. inaquosa*, *M. taraxaci*, and *M. violae* were 35.5%, 36.4%, 35.7% and 36.0%, respectively, values which are below the recommended 70 % cut-off point for the delineation of prokaryotic species (Wayne et al. 1987). Similar results were observed when OrthoANI values were calculated for each pair, obtaining values under 90 % between the new strain and the type strains of *Micromonospora*. These results confirm that the isolate represents a new taxon within the genus *Micromonospora*.

In conclusion, the phylogenetic, chemotaxonomic and morphological data demonstrate that strain S2509T represents a novel species of the genus *Micromonospora*, for which the name *Micromonospora orduensis* sp. nov. is proposed.

Description of *Micromonospora orduensis* sp. nov.

Micromonospora orduensis (or.du.en'sis. N.L. fem. adj. orduensis of or belonging to Ordu, from where the type strain was isolated).

Aerobic, Gram-staining positive actinomycete that forms extensively branched and non-fragmenting substrate mycelium. Aerial hyphae are absent. Spores are single, non-motile and have smooth surfaces. Good growth is observed on ISP2, ISP3, modified Bennett's and tryptic soy agar; moderate growth is observed on ISP6 and nutrient agar; poor growth is observed on ISP4, ISP5, ISP7 and Czapek's agar. The colour of the colonies varies from light orange to dark brown. Growth occurs from pH 5.0 to 11, with an optimum at pH 7.2, and from 28 to 40 °C, with an optimum at 30 °C. The maximum NaCl concentration for growth is 3 % (w/v). Nitrate reduction is positive. Arbutin and aesculin are hydrolysed, but not allantoin or urea. Tween 40 and Tween 80 are degraded, but not adenine, casein, guanine, gelatin hypoxanthine, xanthine or xylan. D-Arabinose, L-arabinose, D-cellobiose, dextrin, D-fructose, D-galactose, D-lactose, D-mannose, D-maltose, L-rhamnose, D-ribose,

D-sucrose and D-xylose are utilised as sole carbon sources but adonitol, dextran, inuline, D-mannitol, L-sorbose, D-sorbitol, xylitol are not. α -Isoleucine, L-alanine, L-arginine, glycine, L-cysteine, L-hydroxyproline, L-methionine, L-phenylalanine, L-proline, L-serine, L-valine and are utilised as sole nitrogen sources, but L-histidine and L-tyrosine are not. The characteristic whole cell sugars are arabinose, glucose, rhamnose, ribose and xylose. The predominant menaquinones are MK-9(H8), MK-9(H6), MK-10(H8), MK-9(H4), MK-10(H4) and MK-10(H6). The main polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol, and the main components of the cellular fatty acids are iso-C16:0, iso-C15:0 and 10-methyl C17:0. The G+C content of the genomic DNA of the type strain is 71.7 mol %.

The type strain, S2509T (=DSM 45926T = KCTC 29201T), was isolated from a Black Sea sediment sample collected along Melet River offshore of the southern Black Sea coast, Ordu, Turkey. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and the genome of strain S2509T are KF494805 and VDFY000000000, respectively.

Acknowledgements

AV is gratefully acknowledge support from Ondokuz Mayıs University (project no. PYO.FEN. 1901.12.014) and the School of Biology (Newcastle University). LC thanks the University of Salamanca for a postdoctoral fellowship. Genome sequencing was provided by MicrobesNG (<http://www.microbesng.uk>), which is supported by the BBSRC (grant number BB/L024209).

Author contributions

AV, NS and HPK designed the study. AV isolated the strain and carried out phenotypic characterisation and single gene phylogenies. LC carried out chemotaxonomic analysis and genome sequencing and analysing. DC carried out scanning electron microscopy analysis and JMI carried out fatty acids determination. AV and LC wrote the manuscript. All authors have revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants and/or animals performed by any of the authors. The formal consent is not required in this study.

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Table 1. Phenotypic properties of strain S2509^T and the closely related type strain *M. chokoriensis* DSM 45160^T. Strains: **1**, S2509^T; **2**, *M. chokoriensis* DSM 45160^T. (+) positive, (-) negative.

Characteristics	1	2
Major menaquinones (> 10%)	MK-9(H ₈) MK-9(H ₆) MK-10(H ₈) MK-9(H ₄) MK-10(H ₄) MK-10(H ₆)	MK-10(H ₄) ^a
Polar lipids profile	DPG, PE, PI	DPG, PE, PG, PI, PIMs ^a
Major fatty acid (>10.0 %)	<i>iso</i> -C _{16:0} , <i>iso</i> -C _{15:0} , 10- <i>methyl</i> C _{17:0}	<i>iso</i> -C _{15:0} , <i>iso</i> - C _{17:0} , C _{17:0}
Whole-cell sugar	Ara, glu, rha, rib, xyl	Ara, gal, glu, man, rib, xyl ^a
Growth temperature range °C	28-40	20-37
pH range for growth	5.0-11.0	5.0-9.0
Biochemical Tests		
Nitrate Reduction	+	-
Hydrolysis of Tween 40	+	-
Hydrolysis of Tween 80	+	-
pH tolerance		
10	+	-
11	+	-
Temperature		
20 °C	-	+
40 °C	+	-
Use as sole carbon source (1.0 %, w/v)		
D-Mannitol	-	+
Use as sole nitrogen source (0.1 %, w/v)		
L-Hydroxyproline	+	-
L-Methionine	+	-
L-Tyrosine	-	+

Abbreviations: +, positive; -, negative; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; Ara, arabinose; Gal, galactose; Glu, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose. Data taken from ^aAra and Kudo (2007)

Table 2. Cellular fatty acid composition of strain S2509^T and the closely related type strain *M. chokoriensis* DSM 45160^T.

Strains: 1, S2509^T; **2,** *M. chokoriensis* DSM 45160^T. All cellular fatty acid composition data was determined in this study.

Fatty acids	1	2	378
Saturated			38
C _{15:0}	-	3.3	-
C _{16:0}	1.0	1.4	-
C _{17:0}	5.5	17.2	384
C _{18:0}	1.3	-	38
C _{19:0}	0.3	-	38
Saturated branched			388
<i>iso</i> -C _{14:0}	1.2	-	38
<i>iso</i> -C _{15:0}	17.0	42.2	391
<i>iso</i> -C _{16:0}	28.1	7.3	39
<i>iso</i> -C _{17:0}	4.9	11.5	39
<i>iso</i> -C _{18:0}	0.6	-	395
<i>anteiso</i> -C _{15:0}	5.0	7.6	39
<i>anteiso</i> -C _{17:0}	7.9	3.2	35
Unsaturated straight			40
C _{17:1} W8c	5.9	-	-
C _{17:1} W9c	0.4	-	402
C _{18:1} W9c	2.1	-	40
Unsaturated branched			406
<i>iso</i> -C _{15:1} G	0.1	-	406
<i>iso</i> -C _{16:1} H	0.9	-	40
C _{18:1} 2OH	0.1	-	409
10-methyl C _{17:0}	12.0	2.0	41
10-methyl C _{18:0} , TBSA	2.3	-	41
*Summed features			413
3	0.3	-	41
6	0.6	-	416
9	2.7	-	-
			41
			419

*Summed features: 3 (C_{16:1}W7c/C_{16:1}W6c; C_{16:1}W6c/C_{16:1}W7c), 6 (C_{19:1}W11c/C_{19:1}W9c; C_{19:1}W9c/C_{19:1}W11c), 9 (10-methylC_{16:0}/*iso*-C_{17:1}W9c)

Fig. 1.

Fig.2.

Fig. 3.

Figure captions

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain S2509^T and amongst its phylogenetic neighbours. Asterisks (*) indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) tree-making algorithms. Numbers at the nodes indicate the levels of bootstrap support (%); only values over ≥ 50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per site.

Fig. 2. Neighbour-joining phylogenetic tree based on 1131 bp *gyrB* gene sequences of strain S2509^T within the genus *Micromonospora*. Asterisks (*) indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) tree-making algorithms. *Catellatospora citrea* DSM 44097^T (Ga0197484_11) was used as an outgroup. Bootstrap percentages based on 1000 replicates are shown; only values over ≥ 50 % are shown. Bar, 0.02 substitutions per nucleotide position.

Fig. 3. Whole-genome sequence tree generated with TYGS for strain S2509^T and closely related species of the genus *Micromonospora*. Tree inferred with FastME from GBDP distances calculated from genome sequences. Branch lengths are scaled in terms of GBDP distance formula d5; numbers above branches are GBDP *pseudo*-bootstrap support values from 100 replications.

Supplementary Fig. S1. Scanning electron micrograph of strain S2509^T grown on N-Z-Amine (DSMZ-medium 554) medium at 30 °C for 43 days.

Supplementary Fig. S2. The phospholipids of strain S2509^T. Diphenylphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), glycolipid (GL) and two unidentified phospholipids (PL₁ - PL₂). a, Using dragendorff's reagent; b, Using ninhydrin

reagent; c, Using molybdenum blue reagent; d, Using molybdate phosphoric acid reagent. Solvents used were CHCl₃: CH₃OH: distilled water (65:25:4) for the first dimension and CHCl₃: glacial acetic acid: CH₃OH: distilled water (80:12:15:4) for the second dimension.

Supplementary Fig. S3. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain S2509^T and amongst its phylogenetic neighbours. Asterisks (*) indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) tree-making algorithms. *Catellatospora citrea* DSM 44097^T (X93197) was used as out group. Numbers at the nodes indicate the levels of bootstrap support (%); only values over ≥ 50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per site.

Supplementary Fig. S4. Maximum likelihood tree based on almost complete 16S rRNA gene sequences showing the position of strain S2509^T amongst its phylogenetic neighbours.

Supplementary Fig. S5. Maximum parsimony tree based on almost complete 16S rRNA gene sequences showing the position of strain S2509^T amongst its phylogenetic neighbours.

Supplementary Fig. S6. Neighbour-joining phylogenetic tree based on 1131 bp *gyrB* gene sequences of strain S2509^T within the genus *Micromonospora*. Asterisks (*) indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) tree-making algorithms. *Catellatospora citrea* DSM 44097^T (Ga0197484_11) was used as an outgroup. Bootstrap percentages based on 1000 replicates are shown; only values over ≥ 50 % are shown. Bar, 0.02 substitutions per nucleotide position.

Supplementary Table S1. Cultural characteristics of the novel strain S2509^T and closely related type strain.

Fig S1

DSM 45926

Dragendorff

Ninhydrin

Molybdenum blue

Molybdate phosphoric acid

Fig S3

Fig. S3.

Fig. S4

Fig. S5

Fig S6

Table S1**sTable S1.** Cultural characteristics of the novel strain S2509^T and closely related type strain.

	<i>Micromonospora</i> sp. S2509 ^T		<i>M.chokoriensis</i> DSM 45160 ^T	
Media	Growth	Color of colony*	Growth	Color of colony*
Yeast extract-malt extract agar (ISP 2)	Good	Dark orange	Good	Dark brown
Oatmeal agar (ISP 3)	Good	Dark brown	Moderate	Light orange
Inorganic salt starch agar (ISP 4)	Poor	Light orange	Moderate	Light orange
Glycerol asparagine agar (ISP 5)	Poor	Light orange	Poor	Colorless
Pepton-yeast extract iron agar (ISP 6)	Moderate	Light orange	Poor	Orange yellow
Tyrosine agar (ISP 7)	Poor	Dark orange	Poor	Light yellow
Modified Bennett's agar	Good	Light orange	Good	Dark brown
Czapek's agar	Poor	Light orange	Good	Brown
Nutrient agar	Moderate	Light orange	Poor	Orange yellow
Tryptic soy agar (TSA)	Good	Light orange	Moderate	Light brown

* The ISCC-NBS color charts were used to determine the designations of colony color (Kelly 1964).

No diffusible pigments were observed on any of the tested media