**Streptomyces manganisoli** sp. nov., a novel actinomycete isolated from manganese-contaminated soil

Ping Mo,† Jiarong Zhao,† Kaqin Li,† Xinke Tang,*, and Jian Gao†,2,*

**Abstract**

A novel actinomycete isolate, designated strain MK44T, was isolated from a Manganese-polluted soil sample collected near Xiangtan Manganese Mine, South Central China and subjected to a polyphasic taxonomic characterization. Comparison of 16S rRNA gene sequences showed that strain MK44T was a member of the genus *Streptomyces* and most closely related to *Streptomyces specialis* JCM 16611T (97.9 %) and *Streptomyces mayteni* JCM 16957T (97.4 %). The DNA–DNA relatedness between strain MK44T and the above two related type species were 30.9±0.3 and 29.9±3.5 %, respectively, values which are far lower than the 70 % threshold for the delineation of a novel prokaryotic species. Furthermore, the results of physiological, biochemical and chemotaxonomic tests allowed further phenotypic differentiation. Therefore, it is concluded that strain MK44T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces manganisoli* sp. nov. is proposed. The type strain is MK44T (=GDMCC 4.137T=KCTC 39920T).

In recent decades, soil pollution by heavy metals has become a serious and widespread problem in many parts of the world [1]. Heavy metal contamination of soil may pose risks and hazards to humans and the ecosystem through: direct ingestion or contact with contaminated soil; the food chain; drinking contaminated ground water; reduction in food quality via phytotoxicity; reduction in land usability for agricultural production causing food insecurity; and land tenure problems [2]. Therefore, the development of remediation methods for metal contaminated soils has become an urgent task for protecting public health and the environment. Bioremediation has attracted the attention of scientists and biotechnologists for environmental control and it employs biological agents, such as micro-organisms like yeast, fungi, algae and bacteria, to treat contaminated soils or water. Microbial bioremediation for *in situ* removal of organic pollutants, heavy toxic metals, radionuclides, etc., can be applied successfully [3–7]. There is sparse information on the use of actinomycetes for bioremediation of heavy metals in soils [8–13], but it is still of great significance for us to collect actinomycetes resources from a variety of heavy metal pollution soils.

Recently, hundreds of actinomycetes strains were isolated from alpine wetland and manganese-polluted soils in the Hunan Province of China, one of strains, designated MK44T, was isolated by the dilution-plate method from manganese-polluted soil and identified using a polyphasic taxonomic approach. The results revealed that strain MK44T represents a novel *Streptomyces* species, for which the name *Streptomyces manganisoli* sp. nov. is proposed.

Strain MK44T was isolated from a soil sample of a manganese-polluted area of Heling town (27° 53′–28° 03′ N, 112° 45′–112° 55′ E), in Xiangtan of Hunan province, in South Central China by a dilution plate method on modified proline agar medium [14], supplemented with 2.0–3.0 ml K2Cr2O7 solution (1.775 g l−1) in a 100 ml medium to reduce fungal contamination [15]. Then the strain was purified by restreaking on Gause’s synthetic agar medium no. 1 [16] at 30 °C for 7–12 days. The purified strain was maintained for long-term preservation in a 30 % (w/v) glycerol suspension at −80 °C. The type strains, *Streptomyces specialis* JCM 16611T and *Streptomyces mayteni* JCM 16957T, were purchased from the Japan Collection of Microorganisms (JCM). Reference strains were cultured under the same conditions for comparative testing.

Cell morphology of strain MK44T was observed by light microscope (Olympus BX41) and scanning electron microscope (Philips FEIXL30), using cultures grown on Gause’s synthetic agar medium no. 1 for 7-15d. The cultural
characteristics of strain MK44\(^T\) were determined on various media including Gause’s synthetic medium no. 1, International *Streptomyces* Project (ISP) media 2–7 [17] and nutrient agar prepared as described by Dong and Cai [18]. The colour of colonies and soluble pigments were determined by the methods described by Ridgway [19]. Tolerance to different temperatures (4, 10, 15, 18, 25, 28, 32, 40 and 45 °C) was tested on Gause’s synthetic agar medium no. 1 for 14 days. The temperatures were measured by a thermometer with calibration in water baths. Growth over a range of pH (3, 4, 5, 6, 7, 8, 9, 10 and 11) was investigated in liquid nutrient agar medium at 28 °C for 14 days on a rotary shaker. The pH was adjusted before and after sterilization using sodium hydroxide or hydrochloric acid. The concentrations of NaCl (0, 1, 2, 3, 4, 5, 6 and 7 %, w/v) was tested on liquid medium which contained 3.0 g beef extract, 10.0 g peptone, 1 l distilled water; pH 7.2–7.4. The utilization of carbon and nitrogen sources was determined using methods described previously [17]. Susceptibility to antimicrobial agents was examined by the disc diffusion method [20] using antibiotic-impregnated discs (Hangzhou Microbial Reagent Co.). The following antibiotic discs, with concentration of the drug per disc as stated in parentheses, were used: amikacin (30 µg), ampicillin (10 µg), carbenicillin (100 µg), cefamezine (30 µg), cefoperazone (75 µg), cefadine (30 µg), cefazidime (30 µg), ceftriaxone (30 µg), cefuroxim (30 µg), cephalexin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), compound sulphonamethoxazole (1.25 µg), doxycycline (30 µg), erythromycin (15 µg), furazolidone (300 µg), gentamicin (10 µg), midecamy cin (30 µg), minocycline (30 µg), neomycin (30 µg), norfloxacin (10 µg), ofloxacin (5 µg), oxacillin (1 µg), penicillin (10 U), piperacillin (100 µg), polymyxin B (300 IU), ranamycin (30 µg), tetracycline (30 µg) and vancomycin (30 µg). The following tests were carried out using the methods described by Xu et al.: liquefaction of gelatin, hydrolysis of starch and ascorbic acid, reduction of nitrate, production of H\(_2\)S, and degradation tests for Tween 20, Tween 40 and Tween 80 [21]. The hydrolysis of aesculin was detected according to Ruan et al. [22]. The experiments were carried out in triplicate.

The isomer of diaminopimelic acid analysis and sugar analysis of whole-cell hydrolysates were performed according to the procedures described by Hasegawa et al. [23] and Lechevalier and Lechevalier [24]. Cellular fatty acid composition was tested by growing cells of strain MK44\(^T\), *S. specialis* JCM 16611\(^T\) and *S. mayotoni* JCM 16957\(^T\) on trypticase soy broth medium after incubation for 8 days at 28 °C. The fatty acid methyl esters were prepared according to the protocol of the Sherlock Microbial Identification system (MDI) and analysed by gas chromatography (6968; Hewlett Packard) using the Microbial Identification software package [25]. Menaquinones were extracted according to the method of Collins et al. [26] and were analysed by high-performance liquid chromatography (LC-20A; Shimadzu). The polar lipids were extracted and separated by two-dimensional thin-layer chromatography [27] and analysed on Merck silica gel 60 F\(_{254}\) aluminium-backed thin-layer plates. The plate dotted with sample was subjected to two-dimensional development, with the first solvent of chloroform–methanol–water (65:25:4, v/v/v) followed by the second solvent of chloroform–methanol–acetic acid–water (80:18:12:5, v/v/v/v).

Genomic DNA was extracted using the method described by Weisburg et al. [28]. The 16S rRNA gene was amplified by using universal primers 27F (5’-AGA GTT TGA TCC TGG CTC AG-3’) and 1492R (5’-ACG TTT TCA TAA GCA CCT T-3’) [29]. The PCR product was purified using a MagExtractor PCR and Gel Clean up kit (TOYOBO) according to the manufacturer’s instructions and sequenced directly using an automated DNA sequencing system (ABI 3730XL; Applied Biosystems) by Sangon Biotech (Shanghai). The 16S rRNA gene of strain MK44\(^T\) was compared with public databases and the EzTaxon-e server [30], then the 16S rRNA sequences of strain MK44\(^T\) and 31 closely related type strains were used to reconstruct phylogenetic trees using a software package [31], after alignment using the CLUSTAL_W program [32]. The alignment was manually verified and adjusted before reconstructing phylogenetic trees. The neighbour-joining [33] and maximum-likelihood [34] methods were used to generate trees with 1000 bootstrap replicates. The G+C content of the genomic DNA of strain MK44\(^T\) was determined by the thermal denaturation procedure [35]. The DNA–DNA hybridization experiments were done in triplicate by the initial renaturation rate method [36].

Morphological observation of 14-day-old cultures of strain MK44\(^T\) on Gause’s synthetic agar medium no. 1 revealed that it had typical characteristics of genus *Streptomyces*, and formed well-developed, branched substrate mycelium that differentiated into spiral spore chains consisting of oval-shape or rod-shaped spores with smooth surfaces (Fig. 1). Strain MK44\(^T\) exhibited good growth on ISP2, ISP3, ISP4 and ISP5, moderate growth on ISP 7 and nutrient agar, and no growth on ISP6. No diffusible pigment was observed on all tested media. Detailed cultural characteristics are presented in Table S1 (available in the online version of this article). Strain MK44\(^T\) grew well between pH 5.0 and 9.0, with an optimum pH of 7.0. The range of growth temperature was determined to be 10–40 °C, with the optimum growth temperature being 28–32 °C. Strain MK44\(^T\) could grow in the presence of 0–5 % NaCl (w/v) and optimally at 0 % (w/v). Detailed physiological characteristics are presented in the species description.

The cell wall of strain MK44\(^T\) contained LL-diaminopimelic acid and the whole-cell hydrolysates were ribose, mannosne and glucose. Strain MK44\(^T\) exhibited an unusual quinone system, with MK-10 (H8) (28.7 %), MK-9 (H6) (25.9 %), MK-10 (H6) (23.8 %), MK-9(H6) (12.2 %) and MK-9 (H10) (9.5 %). The predominant fatty acid (>10 %) components of strain MK44\(^T\) were iso C\(_{16:1}\) (14.4 %) and iso-C\(_{16:0}\) (51.3 %). The polar lipids of strain MK44\(^T\) consisted of diphosphatidyl glycerol, phosphatidyl glycerol, phosphatidylinositol mannoside, phosphatidyl inositol and several unidentified phospholipids (Fig. S1). All the chemotaxonomic data are consistent with the assignment of strain MK44\(^T\) to the genus of *Streptomyces*. 

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**Fig. 1.** Morphological characteristics of strain MK44\(^T\) on Gause’s synthetic agar medium no. 1: (A) colony; (B) aerial mycelia with rod-shaped spores; and (C) absence of aerial mycelium.
Fig. 1. Optical micrograph (a) and scanning electron micrograph (b) of strain MK44\(^T\) grown on Gause’s synthetic agar medium no. 1 at 28 °C after incubation for 14 days.

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Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between selected species of the genus Streptomyces. Actinomadura hibisca JCM 9627\(^T\) was used as an outgroup. Bootstrap percentages over 50% derived from 1000 replications are showed at the nodes. Asterisks indicate branches also recovered in the maximum-likelihood tree. Bar, 0.01 nucleotide substitutions per site.
A BLAST search of the 16S rRNA gene sequence from the EzBioCloud database indicated that strain MK44\(^T\) was grouped into the genus *Streptomyces* and exhibited 97.9 % similarity to *S. specialis* JCM 16611\(^T\), 97.4 % similarity to *S. mayteni* JCM 16957\(^T\) and <97.0 % similarity to various other strains. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain MK44\(^T\) clustered with *S. specialis* JCM 16611\(^T\), *S. mayteni* YIM 60475\(^T\), *Streptomyces hainanensis* YIM 47672\(^T\), *Streptomyces sedi* YIM 65188\(^T\), *Streptomyces hoyanensis* S1412\(^T\) and *Streptomyces avicenniae* NRRRL B-24776\(^T\) in the neighbour-joining tree (Fig. 2). This result was also supported by the maximum-likelihood method analysis (Fig. S2). Stackebrandt and Ebers have pointed out that a 16S rRNA gene sequence similarity threshold range of 98.7–99 % is the point at which DNA–DNA reassociation experiments should be mandatory for testing the genomic uniqueness of a novel isolate [37]. Although it was low (<98.7 %) for the 16S rRNA gene sequence similarity between strain MK44\(^T\) and the above two related type species, were 30.9±0.3 and 29.9±3.5 %, respectively, values which are far lower than the 70 % threshold for the delineation of a novel prokaryotic species [38]. In addition, the DNA G+C content of strain MK44\(^T\) was 75.7 mol%, which is within the range 69–78 % of the members of the genus *Streptomyces* [39].

![Image](https://www.microbiologyresearch.org/)

**Table 1.** Differential characteristics of strain MK44\(^T\), *S. specialis* JCM 16611\(^T\) and *S. mayteni* JCM 16957\(^T\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MK44(^T)</th>
<th>JCM 16611(^T)</th>
<th>JCM 16957(^T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH range for growth</td>
<td>5.0–9.0</td>
<td>6.0–10.0</td>
<td>6.0–8.0</td>
</tr>
<tr>
<td>Temperature range for growth</td>
<td>10–40 °C</td>
<td>25–40 °C</td>
<td>18–32 °C</td>
</tr>
<tr>
<td>NaCl tolerance (% w/v)</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Carbon source utilization:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adonitol</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Arabino</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>l-Arabino</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Fructose</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Mannose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Ribose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>d-Rhamnose</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Malose</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Xylobiot</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Nitrogen source utilization:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-Proline</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>l-Tyrosine</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Predominant menaquinones (&gt;10 %)*</td>
<td>MK-10 (H8), MK-10 (H6), MK-9 (H8), MK-9 (H6), MK-9 (H10), MK-10 (H8), MK-9 (H10), MK-10 (H6)</td>
<td>MK-10 (H4), MK-10 (H6), MK-9 (H4), MK-9 (H6)</td>
<td>MK-9 (H8), MK-9 (H10), MK-10 (H6)</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>DPG, PG, PI, PIM, PL</td>
<td>DPG, PG, PI, PE, PIM, PL, AL</td>
<td>DPG, PG, PL, PE</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10 %)</td>
<td>iso H-C(<em>{16:1}), iso C(</em>{16:0})</td>
<td>iso C(<em>{16:0}), anteiso C(</em>{17:0}) C(<em>{18:0}) c, anteiso C(</em>{17:0})</td>
<td>C(_{16:0}), Summed feature 3, Summed feature 8</td>
</tr>
</tbody>
</table>

*Data regarding the component parts of menaquinones and polar lipids for the reference strains from Kämper [40] or Chen [41].

DPG, Diphasphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PIM, phosphatidylinositol mannoside; PL, unidentified phospholipid; AL, unidentified aminolipid; L, unidentified lipids.

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16957T could not. Other phenotypic differences included utilization of adonitol, L-arabinose, D-rhamnose and D-ribose. In addition, some chemotaxonomic characteristics, such as the absence of phosphatidylethanolamine, C16:0 and MK-9 (H4) or the presence of iso-C12:0, C13:0 and iso H-C18:1, could also distinguish it from the reference strains (Tables 1 and S2, Fig. S1). In conclusion, it is evident from the phenotypic and chemotaxonomic data that strain MK44T represents a novel species of the genus Streptomyces, for which the name *Streptomyces manganisoli* sp. nov. is proposed.

**DESCRIPTION OF STREPTOMYCES MANGANISOLI SP. NOV.**


Aerobic, Gram-stain-positive actinomycete that forms well-developed branched substrate hyphae and aerial mycelia that differentiate into spiral spore chains consisting of oval-shaped or rod-shaped spores with smooth surfaces. Growth occurs at pH 5.0–9.0, 10–40 °C and 0–5 % NaCl (w/v). Starch, Tween 40 and Tween 80 are degraded, but aesculin is not. Gelatin liquefaction, H2S production and nitrate reduction are negative. Mellezitose, D-fructose, myo-inositol, D-galactose, D-ribose, xyitol and xylose are utilized as sole carbon sources for growth, but not D-arabinose, L-arabinose, cellobiose, D-sorbitol, D-mannose, lactose, maltose, D-rhamnose, lactose, maltose, sucrose or starch. Glycine, cysteine, L-alanine, L-histidine, D-phenylalanine, L-proline, L-serine, D-valine and L-tyrosine are not utilized as sole nitrogen sources. Strain MK44T is sensitive to minocycline, gentamicin, amikacin, ranamycin, neomycin. The cell wall contains L-diaminopimelic acid. The whole cell hydrolysates are ribose, mannose and glucose. The predominant menaquinones are MK-10 (H8), MK-9 (H4), MK-10 (H6) and MK-9(H6). The major fatty acids components are iso H-C16:1 and iso-C16:0. The polar lipids of strain MK44T contain diphosphatidyl glycerol, phosphatidyl glycerol, phosphatidylinositol mannoside, phosphatidyl inositol and several unidentified phospholipids.

The type strain, MK44T (=GDMCC 4.137T=KCTC 39920T), was isolated from a soil sample of a manganese-polluted area of Heling, in Xiangtan city of Hunan province, in South Central China. The DNA G+C content of strain MK44T is 75.7 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.


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