**Streptomyces lopnurensis** sp. nov., an actinomycete isolated from soil

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A novel actinomycete, designated strain TRM 49590\(^T\), was isolated from a soil sample from Lop Nur in Xinjiang Province, China. Strain TRM 49590\(^T\) was aerobic, Gram-staining-positive, with an optimum NaCl concentration for growth of 1.5\% (w/v) and an optimum temperature for growth of 28–37 °C. The aerial mycelium was sparse, cylindrical and smooth-surfaced with irregular branches on ISP medium 4. The whole-cell sugars of strain TRM 49590\(^T\) were ribose and glucose. The diagnostic diamino acid contained LL-diaminopimelic acid. The predominant menaquinones were MK-9(H\(_6\)) and MK-9(H\(_8\)), with MK-9(H\(_4\)) and MK-10(H\(_6\)) present in smaller amounts. The major fatty acids were iso-C\(_{16:0}\), anteiso-C\(_{15:0}\) and anteiso-C\(_{17:0}\). The G+C content of the genomic DNA was 62.2 mol\%. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and phosphatidylinositol mannoside. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain TRM 49590\(^T\) belongs to the genus *Streptomyces* with a sequence similarity of 97.16\% with the most closely related species *Streptomyces sodiiphilus*. Based on these observations, strain TRM 49590\(^T\) is proposed to represent a novel species of the genus *Streptomyces* for which the name *Streptomyces lopnurensis* sp. nov. is suggested. The type strain is TRM 49590\(^T\) (=CCTCC AA 2013018\(^T\)=NRRL B59108\(^T\)).

The genus *Streptomyces* was initially introduced by Waksman & Henrici (1943), and at the time of writing there are more than 600 species with validly published names. Streptomycetes are ubiquitous micro-organisms living mostly in soil and other environments such as water, salt or alkaline lake sediments, plant surfaces or internal and extreme environments (Lee, H. J. & Whang, K. S. 2014). Members of the genus *Streptomyces* are Gram-positive, aerobic actinomycetes, and have a high DNA G+C content (69–73 mol\%). These species have wide-ranging metabolic pathways and potential applications in the production of antibiotics, vitamins, enzymes, enzyme inhibitors and bioactive compounds of importance to the food, agricultural and pharmaceutical industries (McCarthy & Williams 1992; Lazzarini et al. 2000; Berdy, 2005). Strain TRM 49590\(^T\) was isolated from a soil sample collected from Lop Nur in Xinjiang Province, north-west China. Preliminary results showed that TRM 49590\(^T\) had interesting physiological characteristics; thus, in the present study it was classified further using a polyphasic approach.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TRM 49590\(^T\) is AB932597.

Two supplementary figures are available with the online Supplementary Material.

Strain TRM 49590\(^T\) was isolated from a soil sample collected from Lop Nur in Xinjiang Province, north-west China (39° 35′ N 89° 46′ E). Lop Nur is located in south-eastern Xinjiang, which was once China’s second largest salt water lake. Now Lop Nur has become a large salt crust, due to the influences of water conservancy projects and climate change; it was once also a nuclear test site. Therefore, research into soil micro-organisms of Lop Nur is of great significance. The strain was isolated using the standard dilution plate method and grown on GW1 medium (Guan et al., 2010) at pH 9 and incubated at 37 °C for 1 week. Strain TRM 49590\(^T\) was maintained on the activation medium, ISP medium 4 (Shirling & Gottlieb, 1966) at 4 °C and as a glycerol suspension (20\%, v/v) at −20 °C. Morphological, physiological and biochemical investigations of strain TRM 49590\(^T\) were carried out by following the standard protocols of the International *Streptomyces* Project (Shirling & Gottlieb, 1966, 1968a, b, 1969) and of Williams et al. (1989).

Cultural characteristics were determined after incubation on ISP medium (Kelly, 1964; Shirling & Gottlieb, 1966). Cultural characteristics were recorded on seven standard media after incubation at 37 °C for 14 days. The organism showed poor growth on ISP medium 2, ISP medium 3, ISP
medium 5 and ISP medium 7, with moderate growth on ISP medium 4, ISP medium 1, Czapek’s agar and nutrient agar. There was no soluble pigment produced in any of the media. The morphological characteristics of strain TRM 49590\textsuperscript{T} were observed by light microscopy (Axioskop 20; Zeiss) and scanning electron microscopy (Quanta; FEI) after incubation on ISP medium 4 at 37 °C for 15 days. The colour of aerial hyphae and substrate mycelium was light-yellow on ISP medium 4. The aerial mycelium was sparse, cylindrical and smooth-surfaced with irregular branches. At the end of the aerial mycelium bud-shaped spores formed and sporangia were not found (Fig. 1).

Media and procedures used for the determination of the physiological features and carbon source utilization were as described by Williams \textit{et al.} (1989). Strain TRM 49590\textsuperscript{T} was able to grow between pH 6.0 and pH 11.0 (tested at 1 pH unit intervals), with optimum growth at pH 9.0. The temperature range for growth was 23–38 °C (tested at 2 °C unit intervals) with an optimum temperature of 28–37 °C. Strain TRM 49590\textsuperscript{T} grew in the presence of 0–5 % NaCl (w/v) with optimal growth at 1.5 % (w/v) NaCl. The strain degraded gelatin and milk but not starch or cellulose. It was catalase-positive, but oxidase-negative. It could reduce nitrate, but could not produce hydrogen sulfide. Tweens 20, 40, 60 and 80 were hydrolysed, but adenine, xanthine and hypoxanthine were not. It utilized D-glucose, L-arabinose, trehalose, L-rhamnose, D-ribose, D-mannitol and D-salicylic, but could not utilize D-raffinose and D-sorbitol as the sole carbon source.

Analysis of the diaminopimelic acid isomers and sugars of whole-cell hydrolysates was performed according to the procedures described by Staneck & Roberts (1974) and Hasegawa \textit{et al.} (1983). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin \textit{et al.} (1984). Menaquinones were extracted using the method of Collins (1985) and analysed by HPLC (Groth \textit{et al.}, 1997). Cellular fatty acid composition was determined using the Microbial Identification System (Sherlock version 6.0; MIDI). DNA G+C content was determined by HPLC as described by Tamaoka & Komagata (1984). Whole-cell hydrolysates of strain TRM 49590\textsuperscript{T} contained mainly ribose and glucose. The cell wall contained 1\textit{L}-diaminopimelic acid. The menaquinones were MK-9(H\textsubscript{4}) (10.1 %), MK-9(H\textsubscript{6}) (65.0 %), MK-9(H\textsubscript{8}) (22.4 %) and MK-10(H\textsubscript{4}) (2.5 %). The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylincholine, phosphatidylinositol, phosphatidylinositol mannoside, two unidentified glycolipids and two unidentified phospholipids. The reagents used for detection were ninhydrin reagent, molybdenum blue reagent, Dragendorff’s reagent, 2-naphthol reagent, anisaldehyde reagent and molybdenum phosphate reagent (Figs S1 and S2, available in the online Supplementary Material). The major cellular fatty acids were iso-C\textsubscript{16}:0 (49.8 %), anteiso-C\textsubscript{15}:0 (15.0 %), anteiso-C\textsubscript{17}:0 (10.4 %) and iso-C\textsubscript{15}:0 (6.5 %), while the fatty acids present in smaller amounts (>1 %) were iso-C\textsubscript{17}:0 (2.8 %), C\textsubscript{16}:0 (1.9 %), C\textsubscript{17}:0 (1.8 %), iso-C\textsubscript{14}:0 (2.2 %), anteiso-C\textsubscript{17}:1\textit{O}9c (1.6 %) and iso-C\textsubscript{16}:1 H (1.2 %). The G+C content of the genomic DNA was 62.15 mol%.

Genomic DNA was extracted from the isolate and the 16S rRNA gene sequence was amplified using an established method (Chun & Goodfellow, 1995). The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved by using the EzTaxon-e server (http://www.eztaxon-e.ezcloud.net/; Kim \textit{et al.}, 2012). The 16S rRNA gene sequence was aligned with the published sequences of closely related bacteria using the EzTaxon server 2.1 (Chun \textit{et al.}, 2007). Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1972) algorithms within the MEGA5 program (Tamura \textit{et al.} 2011). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain TRM 49590\textsuperscript{T} falls within the radius of the genus \textit{Streptomycetes} and had the highest sequence similarity to \textit{Streptomycyes sodiiphilus} YIM 80305\textsuperscript{T} (GenBank accession no.AY236339; 97.16 %). All other species of the genus \textit{Streptomycyes} showed lower sequence similarities (≤96.86 %) to strain TRM 49590\textsuperscript{T}. The phylogenetic tree based on the 16S rRNA gene sequence of strain TRM 49590\textsuperscript{T} and the most closely related type strains of species of the genus \textit{Streptomycyes} is shown in Fig. 2. Topologies of phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms were similar to the tree reconstructed by neighbour-joining analysis. All of the above data confirmed that strain TRM 49590\textsuperscript{T} should be assigned to the genus \textit{Streptomycyes}.

Characteristics differentiating strain TRM 49590\textsuperscript{T} from closely related type species in the genus \textit{Streptomycyes} are shown in Table 1. Observations of strain TRM 49590\textsuperscript{T} with an electron microscope showed that the aerial mycelium
Streptomyces lopnurensis sp. nov. is proposed.

On the basis of evidence from this polyphasic taxonomic study, we propose that strain TRM 49590T represents a novel species of the genus Streptomyces, for which the name *Streptomyces lopnurensis* sp. nov. is proposed.

**Description of Streptomyces lopnurensis sp. nov.**

*Streptomyces lopnurensis* (lop.nur.en’sis, N.L. masc. adj. lopnurensis of or belonging to Lop Nur, a lake in Ruoqiang county, north-western China, where the soil sample was collected).

*Aerobic, Gram-staining-positive, non-motile actinobacterium, with irregular branches. Bud-shaped spores are formed at the end of the aerial mycelium; the spore surface is smooth and no sporangia are formed on the aerial mycelium. The colour of aerial and substrate mycelia is light-yellow on ISP medium 4. Grows well on ISP medium 4, ISP medium 1, Czapek’s agar and nutrient agar at 37 °C. Soluble pigment is not formed in any media. Growth occurs at 23–38 °C (optimum, 37 °C). The pH range for growth is between pH 6.0 and pH 11.0 (optimum, pH 9.0) and the NaCl range for growth is 0–5 % (w/v) NaCl.

On the basis of evidence from this polyphasic taxonomic study, we propose that strain TRM 49590T represents a novel species of the genus Streptomyces, for which the name *Streptomyces lopnurensis* sp. nov. is proposed.

**Fig. 2.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain TRM 49590T and the type strains of phylogenetically closely related species of the genus Streptomyces. *Streptacidiphilus albus* DSM 41753T was used as the outgroup. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 70 % are indicated. Bar, 0.005 substitutions per nucleotide position.
Table 1. Morphological, physiological and biochemical characteristics of strain TRM 49590 T and strains of phylogenetically related species of genus Streptomyces

Strains: 1, TRM 49590 T; 2, Streptomyces sodiiphilus YIM 80305 T; 3, Streptomyces harbinensis NEAU-Da3 T; 4, Streptomyces qinglanensis 172205 T. All strains grew on ISP medium 2 and ISP medium 3 at pH 7, 8, 9 and 10, at temperatures of 28 °C and in the presence of 1, 2 and 3 % (w/v) NaCl; they all contained MK-9(H4) and MK-9(H8). All strains were negative for the utilization of sorbitol (0.5 %, w/v) as the sole carbon source and did not grow at pH 4 or 5 or at temperatures of 4 °C or 42 °C. Data in column 1 are from the present study, while the data in columns 2–4 are taken from previous studies (Li et al., 2005; Hu et al., 2012; Liu et al., 2013). +, Positive; −, negative; ND, no data available. The tests for optimum growth temperature, pH and salinity were performed using different media.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycelium</td>
<td>Sparse, irregular branches</td>
<td>Well-developed, not fragmented</td>
<td>Well-developed, not fragmented</td>
<td>Sparse, white</td>
</tr>
<tr>
<td>Spores</td>
<td>Single, smooth surfaced</td>
<td>Chains of spores</td>
<td>Singly, smooth surfaced</td>
<td>Elliptical, short, rod-shaped, smooth</td>
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<tr>
<td>Diffusible pigment</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Optimum temperature for growth (°C)</td>
<td>28 2 C–37</td>
<td>28</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Optimum NaCl concn (% w/v) for growth</td>
<td>1.50</td>
<td>3</td>
<td>0–5</td>
<td>28</td>
</tr>
<tr>
<td>Optimum pH for growth</td>
<td>9–10</td>
<td>9–10</td>
<td>8</td>
<td>28</td>
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<tr>
<td>Cellulose degradation</td>
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<td>ND</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Milk coagulation and milk peptonization</td>
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<td>−</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Degradation of Tweens 20, 40, 60 and 80</td>
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<td>ND</td>
<td>ND</td>
<td>+</td>
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<td>−</td>
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<td>H2O2 production</td>
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<td>ND</td>
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<td>Utilization as sole carbon source</td>
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<td>L-Arabinose</td>
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<td>D-Mannitol</td>
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<td>−</td>
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<tr>
<td>D-Ribose</td>
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<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Predominant cellular fatty acids</td>
<td>iso-C16 : 0, anteiso-C15 : 0, anteiso-C17 : 0</td>
<td>anteiso-C15 : 0, anteiso-C17 : 0, iso-C16 : 0, anteiso-C18 : 0</td>
<td>iso-C16 : 0, iso-C17 : 0, 9C, anteiso-C15 : 0, anteiso-C16 : 0</td>
<td></td>
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</table>

The type strain, TRM 49590 T (= CCTCC AA 2013018 T = NRRL B59109 T), was isolated from a soil sample collected from Lop Nur in Xinjiang province, north-west China. The G + C content of the genomic DNA of the type strain is 62.2 mol%.

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References


