Streptomyces indoligenes sp. nov., isolated from rhizosphere soil of Populus euphratica

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A novel actinobacterium, designated TRM 43006T, was isolated from the rhizosphere soil of Populus euphratica in Xinjiang Province, north-west China. Phylogenetic and phenotypic analysis demonstrated that strain TRM 43006T belongs to the genus Streptomyces. The strain was aerobic and Gram-stain-positive; the aerial mycelium branched monopodially, forming chains of arthrospores. The spores were oval to cylindrical with smooth surfaces. The whole-cell sugar pattern of strain TRM 43006T consisted of xylose, mannitol, galactose and ribose. The menaquinones were MK-9(H4), MK-9(H6) and MK-9(H10). The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol mannosides and four unknown phospholipids. Major fatty acids were iso-C16:0, iso-C16:1, iso-C14:0 and anteiso-C15:0. The G+C content of the genomic DNA was 69.0 mol%. Comparative 16S rRNA gene sequence analysis indicated that strain TRM 43006T was phylogenetically most closely related to Streptomyces roseoalnicus NBCR 12815T (98.3 % similarity) and Streptomyces sudanensis SD 504T (98.6 %); however, DNA–DNA hybridization studies between S. roseoalnicus NBCR 12815T, S. sudanensis SD 504T and TRM 43006T showed only 30.28 and 30.65 % relatedness, respectively. Based on the evidence from this polyphasic study, strain TRM 43006T represents a novel species of the genus Streptomyces, for which the name Streptomyces indoligenes sp. nov. is proposed. The type strain is TRM 43006T (=KCTC 39611T=CCTCC AA 2015010T).

The genus Streptomyces was first described by Waksman & Henrici (1943). At the time of writing, the genus Streptomyces consists of 778 species with validly published names (http://www.bacterio.net/streptomyces.html) (Euzeby, 2014). Species of the genus Streptomyces have distinct features, such as Gram-stain-positive cell walls, high DNA G+C contents (Manfio, 1995; Williams et al., 1983), the presence of L-diaminopimelic acid in the cell-wall peptidoglycan, but no characteristic sugars of wall chemotype I (sensu Lechevalier, 1970). There is great interest in the members of this genus due to their potential applications in the production of antibiotics, vitamins and enzymes with importance in industry and agriculture (Lazzarini et al., 2000; McCarthy & Williams, 1992). In this study, we report on the classification and characterization of strain TRM 43006T, which was isolated from rhizosphere soil of Populus euphratica in Xinjiang, and which can produce indole derivatives (Sun, 2015). We performed a polyphasic taxonomic on this strain, and propose that it represents a novel species of the genus Streptomyces.

Strain TRM 43006T was isolated from the rhizosphere soil of Populus euphratica collected from Alar, Xinjiang Province, north-west China (40°22′ N 80°30′ E). The sample was isolated on Gause’s synthetic agar (Gause, 1966) at 37°C for 2 weeks. Morphological observations of spores and mycelia were conducted by scanning electron microscopy (Hitachi High-Technologies) of cultures on Gause’s plates incubated at 28°C for 7 days. Carbon-source utilization tests were performed according to the methods described by Shirling & Gottlieb, 1966, and using the basal medium recommended by Pridham & Gottlieb (1948). Growth at various NaCl concentrations (0–10 % (w/v) (0, 1, 3, 5, 8 and 10 %) and different temperatures (5–55 °C) (5, 10, 15, 20, 25, 30, 37, 40, 45, 50 and 55 °C) was examined by growing the strain on Gause’s plates as the basal medium. Growth was tested over pH 4–10 (pH 4, 5, 6, 7, 8,
9 and 10) as described by Xu et al., 2005 on Gause’s medium. Biomass for chemical and molecular studies was obtained by cultivation in Gause’s medium on a shaker at 220 r.p.m. and 37 °C for 3 days. The other physiological characteristics of strain TRM 43006T were assessed by using the media and methods of Gordon et al. (1974). The aim of this study was to identify the exact taxonomic status of strain TRM 43006T by using a polyphasic approach.

Investigations of 7-day-old cultures of strain TRM 43006T revealed that branched substrate mycelium and aerial hyphae were produced. At maturity, the aerial mycelium branched monopodially, forming chains of arthrospores with more than 10 spores per chain (Fig. 1). The spores were straight, sometimes flexuous. The spores were oval to cylindrical in shape with smooth surfaces. The colonies were grey on some media tested (ISP 2, Czapek’s agar, nutrient and Gause’s) and the strain produced yellowish aerial mycelium in ISP 4 medium. Growth was good on all the media. No diffusible pigments were produced on any of the media tested. The temperature and pH ranges for growth were 20–45 °C and pH 6–10, with optimal growth at 37 °C and pH 7.0–8.0. The NaCl concentration for growth was 0–5 % (w/v), with optimal growth at 0 %. Other physiological characteristics of strain TRM 43006T are given in the species descriptions and in Table 1.

Standard procedures were used to determine the type of amino acid in cell-wall hydrolysates and of cell-wall sugars (Hasegawa, 1983). Menaquinones were extracted according to the method of Collins (1980) and analysed by HPLC (Groth et al., 1997). Polar lipids were extracted, examined by two-dimensional TLC and identified with 10 % ethanolic molybdophosphoric acid using the procedures of Minnikin et al. (1984). The cellular fatty acid composition was determined as described by Kämpfer (1996) using the Microbial Identification System (MIDI Sherlock version 6.0). The DNA G+C content of strain TRM 43006T was determined by using the HPLC method (Mesbah, 1989).

Strain TRM 43006T contained ll-diaminopimelic acid as the cell-wall diamino acid, and whole-cell hydrolysates contained mainly ribose, xylose, mannitol and galactose. The menaquinones of strain TRM 43006T were MK-9(H8) (43.1 %), MK-9(H6) (55.0 %) and MK-9(H10) (1.9 %). The major cellular fatty acids were iso-C16:0 (38.6 %), iso-C15:0 (3.3 %), anteiso-C17:0 (7.2 %), C16:0 (4.6 %), iso-C15:0 (3.4 %), anteiso-C17:0 (2.6 %), anteiso-C17:1ω9c (2.2 %), C18:1ω9c (2.0 %) and C17:0 cyclo (1.8 %). The polar lipid profile comprised diphosphatidyglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol and mannosides and four unknown phospholipids (Fig S1, available in the online Supplementary Material). The G+C content of strain TRM 43006T was 69.0 mol%.

Genomic DNA of strain TRM 43006T was extracted from cells grown on Gause’s for 3 days at 37 °C and used as a template for subsequent PCR amplification. Amplification and sequencing of the 16S rRNA gene were performed as described by Kim et al., (2000). Multiple alignments with sequences from the most closely related members of the genus Streptomyces and calculations of sequence similarity were carried out using the EzTaxon-e server (Kim et al., 2012). Phylogenetic analyses were performed by MEGA version 6 (Tamura et al., 2013) using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Mount, 2008) algorithms. For the neighbour-joining method, evolutionary distance matrices were calculated using the algorithm of the Kimura two-parameter model (Kimura, 1980), using SeaView version 4.2 (Gouy et al., 2010). For maximum-likelihood analysis, the best model (JTT+I+G) was picked via the program ProtTest 3 (Darriba et al., 2011). All phylogenetic trees were evaluated by the bootstrap resampling method of Felsenstein (1992) with 1000 replications. As the topologies of these trees were similar (Figs S2 and S3), only the neighbour-joining tree is shown (Fig. 2).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain TRM 43006T fell within the radius of the genus Streptomyces and had the highest sequence similarity to Streptomyces roseolilacinus NBRC 12815T (GenBank accession no. AB184167; 98.6 %). Phylogenetic analysis also showed that strain TRM 43006T fell within a distinct clade with S. roseolilacinus NBRC 12815T and S. sudanensis SD 504T (Fig. 2). The topologies of phylogenetic trees built using the maximum-likelihood (Fig. S2) and maximum-parsimony (Fig. S3) algorithms were similar to the tree reconstructed by neighbour-joining analysis. To determine genomic relatedness, DNA–DNA hybridization was performed using the method of Ezaki & Yabuuchi (1989). Strain TRM 43006T exhibited 30.28 and 30.65 % DNA–DNA relatedness with S. roseolilacinus NBRC 12815T and S. sudanensis SD 504T, respectively. These values were below the threshold value of 70 % recommended by Wayne

**Fig. 1.** Scanning electron micrograph of strain TRM 43006T grown on Gause’s plates at 37 °C for 7 days. Bar, 10 μm.
et al. (1987) for delineating bacterial species. All the above data confirmed that strain TRM 43006^T should be assigned to anovel species of the genus Streptomyces.

In conclusion, strain TRM 43006^T exhibited some chemo-
taxonomic differences from members of the genus Streptomyces: it contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannosides and four unknown phospholipids as diagnostic phospholipids. (Fig. S1), in contrast to data for S. roseolilacinus NBRC 12815^T and S. sudanensis SD 504^T; it had MK-9(H_4), MK-9(H_6), and MK-9(H_8) as the menaquinones, which is distinctly different from S. roseolilacinus NBRC 12815^T and S. sudanensis SD 504^T. Strain TRM 43006^T contained iso-C_{16:0}, iso-C_{16:1}, iso-C_{14:0}, anteiso-C_{15:0}, and anteiso-C_{17:0} as major cellular fatty acids. The G+C content of strain TRM4300^T was 69.0 mol %, which is distinctly different from S. roseolilacinus NBRC 12815^T (Han et al., 2015) and S. sudanensis SD 504^T (Quintana et al., 2008), the nearest neighbouring species. These characteristics indicate that strain TRM 43006^T should be classified as representing a novel species, for which the name Streptomyces indoligenes sp. nov. is proposed.

**Description of Streptomyces indoligenes sp. nov.**

*Streptomyces indoligenes* [in.do.li'ge.nes. N.L. neut. n. *indol-*um indole; N.L. suff. -genes (from Gr. v. *genna* to produce); N.L. adj. *indoligenes* indole-producing].

Aerobic, Gram-stain-positive, non-motile actinobacterium that forms extensively branched substrate mycelium and aerial hyphae. The vegetative mycelium is well developed and irregularly branched. Branched yellowish substrate mycelia fragment into short or elongated rods. Forms white to yellowish aerial mycelia, which are

**Table 1. Differential characteristics between strain TRM 43006^T and the most closely related Streptomyces species**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range for growth (°C)</td>
<td>20–45</td>
<td>10–45</td>
<td>15–45</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>6–10</td>
<td>6–12</td>
<td>5–10</td>
</tr>
<tr>
<td>Mycelia colour</td>
<td>White grey</td>
<td>White</td>
<td>Light yellow</td>
</tr>
<tr>
<td>NaCl for growth (% w/v) (optimum)</td>
<td>0–5 (0)</td>
<td>0–6 (0)</td>
<td>0–6 (0)</td>
</tr>
<tr>
<td>Production of catalase</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H_2S production</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cellulose degradation</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lipase</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of carbon source:</td>
<td></td>
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</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Raffinose</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sole nitrogen source:</td>
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<tr>
<td>L-Valine</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Major cell-wall diamino acid</td>
<td>LL-DAP</td>
<td>meso-DAP</td>
<td>LL-DAP</td>
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<tr>
<td>Major whole-cell sugars</td>
<td>Ribose, xylose,</td>
<td>Ribose, xylose,</td>
<td>Ribose, xylose,</td>
</tr>
<tr>
<td></td>
<td>mannitol, galactose</td>
<td>galactose, glucose</td>
<td></td>
</tr>
<tr>
<td>Phospholipids</td>
<td>DPG, PE, PI, PC, PIM</td>
<td>DPG, PE, PC, NPG, PI</td>
<td>DPG, PE, PC, NPG,</td>
</tr>
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<td>Predominant cellular fatty acids</td>
<td>iso-C_{16:0}, iso-C_{16:1}, iso-C_{14:0},</td>
<td>iso-C_{16:0}, iso-C_{16:1}, anteiso-C_{15:0}, anteiso-C_{17:0},</td>
<td>iso-C_{16:0}, iso-C_{16:1}, anteiso-C_{15:0}, anteiso-C_{17:0},</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>MK-9(H_2), MK-9(H_3), MK-9(H_4)</td>
<td>MK-9(H_2), MK-9(H_3), MK-9(H_4)</td>
<td>MK-9(H_2), MK-9(H_3), MK-9(H_4)</td>
</tr>
</tbody>
</table>

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![Phylogenetic tree](image-url)

**Fig. 2.** Phylogenetic tree showing the relationship between strain TRM 43006\(^T\) and near neighbours based on 16S rRNA gene sequences. The tree was reconstructed by the neighbour-joining method from evolutionary distances calculated. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 70\% are shown. *Kitasatospora setae* NBRC 14216\(^T\) and *Streptacidiphilus albus* DSM 41753\(^T\) were used as the outgroup. Bar, 0.01 nucleotide substitutions per site.

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession No.</th>
<th>Bootstrap Support (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces scabiei</em> ATCC 49173(^T)</td>
<td>(AB026199)</td>
<td>92</td>
</tr>
<tr>
<td><em>Streptomyces europaesiacaebii</em> KACC 20186(^T)</td>
<td>(AY207598)</td>
<td>99</td>
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<tr>
<td><em>Streptomyces decannensis</em> DAS-139(^T)</td>
<td>(EF219459)</td>
<td>87</td>
</tr>
<tr>
<td><em>Streptomyces diastatochromogenes</em> ATCC 12309(^T)</td>
<td>(D63867)</td>
<td>72</td>
</tr>
<tr>
<td><em>Streptomyces caeruleus</em> GIMN4.002(^T)</td>
<td>(GJ329712)</td>
<td>69</td>
</tr>
<tr>
<td><em>Streptomyces rishiriensis</em> NBRC 13407(^T)</td>
<td>(AB184383)</td>
<td>97</td>
</tr>
<tr>
<td><em>Streptomyces sudanensis</em> SD 504(^T)</td>
<td>(EF515876)</td>
<td>72</td>
</tr>
<tr>
<td><em>Streptomyces roseolilacinus</em> NBRC 12815(^T)</td>
<td>(AB184167)</td>
<td>97</td>
</tr>
<tr>
<td><em>Streptomyces indoligens</em> TRM43006(^T)</td>
<td>(KU195301)</td>
<td>72</td>
</tr>
<tr>
<td><em>Streptomyces lirudis</em> NBRC 12793(^T)</td>
<td>(AB184150)</td>
<td>64</td>
</tr>
<tr>
<td><em>Streptomyces gobitricini</em> NBRC 15419(^T)</td>
<td>(AB184666)</td>
<td>47</td>
</tr>
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<td><em>Streptomyces lavendofoliae</em> NBRC 12882(^T)</td>
<td>(AB184217)</td>
<td>45</td>
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<tr>
<td><em>Streptomyces sanysensis</em> 21982(^T)</td>
<td>(FJ261968)</td>
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<tr>
<td><em>Streptomyces purpureus</em> NBRC 13927(^T)</td>
<td>(AB184547)</td>
<td>43</td>
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<tr>
<td><em>Streptomyces peucetius</em> JCM 9920(^T)</td>
<td>(AB045887)</td>
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<tr>
<td><em>Streptomyces kurssanovii</em> NBRC 13192(^T)</td>
<td>(AB184325)</td>
<td>99</td>
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<tr>
<td><em>Streptomyces xantholiticus</em> NBRC 13354(^T)</td>
<td>(AB184349)</td>
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<tr>
<td><em>Streptomyces hundangensis</em> MBRL 251(^T)</td>
<td>(JN560157)</td>
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<td><em>Streptomyces noboritoensis</em> NBRC 13065(^T)</td>
<td>(AB184287)</td>
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<tr>
<td><em>Streptomyces crystallinus</em> NBRC 15401(^T)</td>
<td>(AB184652)</td>
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<tr>
<td><em>Kitasatospora setae</em> NBRC 14216(^T)</td>
<td>(AP010968)</td>
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<tr>
<td><em>Streptacidiphilus albus</em> DSM 41753(^T)</td>
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</table>

**Acknowledgements**

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sometimes branched. The aerial mycelium forms long chains of spores with more than 10 spores per chain and spores are non-motile. The spore chains are normally straight, sometimes flexuous. Growth occurs at 20–45 °C (optimum, 37 °C). The pH range for growth is between pH 6 and pH 10 (optimum, pH 7.5). Growth occurs in the presence of 0–5 % (w/v) NaCl (optimum 0 %). Good growth occurs on all tested media (ISP 2, ISP 4, Czapek’s agar, nutrient agar and Gause’s agar at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 70 % are shown. Kitasatospora setae NBRC 14216\(^T\) and Streptacidiphilus albus DSM 41753\(^T\) were used as the outgroup. Bar, 0.01 nucleotide substitutions per site.

and production of H\(_2\)S. The diagnostic phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannosides and four unknown phospholipids. The predominant menaquinones are MK-9(\(H_6\)), MK-9(\(H_8\)) and MK-9(\(H_{10}\)). Major cellular fatty acids are iso-C\(_{16:0}\), iso-C\(_{16:1}\) and iso-C\(_{14:0}\).

The type strain, TRM 43006\(^T\) (=KCTC 39611\(^T\)=CCTCC AA 2015010\(^T\)), was isolated from rhizosphere soil of *Populus euphratica* in Xinjiang Province, north-west China. The G+C content of the DNA of the type strain is 69.0 mol%.

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