Streptomonospora halotolerans sp. nov., an actinomycete isolated from soil

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A novel actinomycete, designated strain NEAU-Jh2-17ᵀ, was isolated from muddy soil collected from a riverbank in Jilin Province, northern China, and characterized using a polyphasic approach. The 16S rRNA gene sequence of strain NEAU-Jh2-17ᵀ showed highest similarity to those of Streptomonospora nanhaiensis 12A09ᵀ (99.26 %), Nocardiopsis rosea YIM 90094ᵀ (97.31 %), Streptomonospora halophila YIM 91355ᵀ (97.24 %) and Streptomonospora arabica S186ᵀ (97.02 %). Phylogenetic analysis based on 16S rRNA gene sequences demonstrated that strain NEAU-Jh2-17ᵀ fell within a cluster consisting of the type strains of species of the genus Streptomonospora and formed a stable clade with S. nanhaiensis 12A09ᵀ in trees generated with two algorithms. Key morphological and chemotaxonomic properties also confirmed the affiliation of strain NEAU-Jh2-17ᵀ to the genus Streptomonospora. The cell wall contained meso-diaminopimelic acid as the diagnostic diamino acid and whole-cell hydrolysates contained glucose, ribose and galactose. The polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM), two unknown phospholipids (PLs) and two unknown glycolipids (GLs). The predominant menaquinones were MK-10(H₂), MK-10(H₄), MK-10(H₆) and MK-10(H₈). Major fatty acids were C₁₈ : 0 10-methyl, anteiso-C₁₇ : 0, C₁₆ : 0 10-methyl, iso-C₁₅ : 0, C₁₇ : 0 10-methyl and C₁₈ : 0. The DNA G + C content was 71.82 mol%. However, a combination of DNA–DNA hybridization results and some phenotypic characteristics demonstrated that strain NEAU-Jh2-17ᵀ could be distinguished from its closely related relatives. Therefore, strain NEAU-Jh2-17ᵀ is considered to represent a novel species of the genus Streptomonospora, for which the name Streptomonospora halotolerans sp. nov. is proposed. The type strain is NEAU-Jh2-17ᵀ (=CGMCC 4.7218ᵀ = JCM 30347ᵀ).

The genus Streptomonospora, belonging to the family Nocardiopsaceae (Stackebrandt et al., 1997), was established by Cui et al. (2001) (with Streptomonospora salina as the type species) to accommodate a group of strictly halophilic filamentous actinomycetes forming a distinct branch in the 16S rRNA gene phylogenetic tree adjacent to the genera Nocardiopsis and Thermobifida. Subsequently, the description of the genus Streptomonospora was emended by Li et al. (2003) and Zhang et al. (2013) on the basis of whole-cell hydrolysates, polar lipid pattern, menaquinone composition, fatty acids and DNA G + C content. Members of the genus Streptomonospora are characterized by several chemical markers: the diagnostic diamino acid of the peptidoglycan is meso-diaminopimelic acid; whole-cell hydrolysates contain galactose; the phospholipid pattern is complex, with diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol present in all recognized species of the genus except S. salina.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-Jh2-17ᵀ is KM000838.

Two supplementary figures are available with the online Supplementary Material.
Strain NEAU-Jh2-17T was isolated using the standard dilution plate method and was grown on humic acid-vitamin agar (Shirling & Gottlieb, 1966), and maintained by repeated subculture. Whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985). Extracts were analysed by an HPLC-UV method (Wu et al., 1989) using an Agilent Extend-C18 column (150 × 4.6 mm, i.d. 5 μm) at 270 nm. The mobile phase was acetonitrile/isopropl alcohol (60 : 40, v/v). To determine cellular fatty acid compositions, strain NEAU-Jh2-17T was cultivated in SY broth in shake flasks at 28 °C for 14 days. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) and analysed by GC-MS using the method of Xiang et al. (2011).

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence was carried out using a standard procedure (Kim et al., 2000). The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). The almost full-length 16S rRNA gene sequence of strain NEAU-Jh2-17T (1527 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X 1.83 software. The alignment was manually verified and adjusted before reconstruction of phylogenetic trees. The phylogenetic trees reconstructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA software version 5.05 (Tamura et al., 2011) were based on a comparison of 1366 nt present in all of the strains, omitting unaligned regions. The stability of the topology of the phylogenetic tree was assessed using the bootstrap method with 1000 repetitions (Felsenstein, 1985). A distance matrix was generated using Kimura’s two-parameter model (Kimura, 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). 16S rRNA gene sequence similarities between strains were calculated on the basis of pairwise alignment using the EzTaxon-e server (Kim et al., 2012).
The G+C contents of the genomic DNA were determined using the thermal denaturation (Tm) method (Mandel & Marmur, 1968) with *Escherichia coli* JM109 DNA used as the control. DNA–DNA relatedness tests between strain NEAU-Jh2-17T and *S. nanhaiensis* 12A09T, *Nocardiopsis rosea* YIM 90094T, *S. halophilia* YIM 91355T and *S. arabica* DSM 45083T were carried out as described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6×6 multiecell changer and a temperature controller with in-situ temperature probe (Varian). The concentration and purity of DNA samples were determined by measuring the optical density at 260, 280 and 230 nm. The DNA samples used for hybridization were diluted to an OD260 of about 1.0 using 0.1 × saline sodium citrate (SSC) buffer, then sheared using a JY92-II ultrasonic cell disruptor (ultrasonic time 3 s, interval time 4 s, 90 times). DNA renaturation rates were determined in 2× SSC buffer at 70 °C. The experiments were performed with three replications and the DNA–DNA relatedness values were expressed as the mean of the three values.

Morphological observation of a 4-week culture of strain NEAU-Jh2-17T grown on ISP 3 medium revealed that it shared the same morphological characteristics as members of the genus *Streptomonospora*. The aerial mycelium was well developed but not fragmented; at maturity, it formed short chains of oval to cylindrical spores with smooth surfaces (0.4–0.5 × 0.9–1.0 μm), which were straight to flexuous (Fig. 1a). The substrate mycelium was abundant and non-fragmented. Single, oval spores with wrinkled surfaces were borne on sporophores of substrate mycelium (Fig. 1b). Both types of spores were non-motile. Strain NEAU-Jh2-17T exhibited good growth on ISP 2, 3, 5 and 7, Czapek’s agar and nutrient agar, moderate growth on ISP 6 agar, but no growth on ISP 4 agar. White aerial mycelium was produced on ISP 3 and 5 agar, but no aerial mycelium was formed on ISP 2, 4, 6 and 7, Czapek’s agar or nutrient agar. The substrate mycelium colour was yellowish white on ISP 3, 4, 5, 6 and 7 media, pale greenish yellow on ISP 2 medium, brilliant orange yellow on nutrient agar and brilliant yellow on Czapek’s agar. No diffusible pigment was observed on any of the media tested. The isolate grew well between pH 7.0 and 9.0, with an optimum pH of 8.0. The range of temperature for growth was 20–37 °C, with the optimum growth temperature being 28 °C. Strain NEAU-Jh2-17T grew in the presence of 0–10 % (w/v) NaCl. Detailed physiological characteristics are presented in the species description.

The cell-wall hydrolysates of strain NEAU-Jh2-17T contained meso-diaminopimelic acid as the diagnostic diamino acid. Whole-cell hydrolysates contained glucose, ribose and galactose. The polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidyldiglycerol (PG), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM), two unknown phospholipids (PLs) and two unknown glycolipids (GLs) (Fig. S1, available in the online Supplementary Material). The menaquinones were MK-10(H2) (20.2 %), MK-10(H4) (19.7 %), MK-10(H6) (19.4 %), MK-10(H8) (15.9 %) and small amounts of MK-11(H4) (4.8 %), MK-11(H6) (4.6 %), MK-10(H0) (4.2 %), MK-9(H8) (4.2 %), MK-9(H6) (3.9 %) and MK-11(H4) (3.1 %). The cellular fatty acid profile was composed of C18 : 0 10-methyl (20.9 %), anteiso-C17 : 0 (16.2 %), C16 : 0 10-methyl (11.7 %), iso-C15 : 0 (11.3 %), C17 : 0 10-methyl (10.3 %), C18 : 0 (10.0 %), C18 : 1ω7c (4.5 %), iso-C17 : 0 (4.5 %), C17 : 0 (2.7 %), iso-C14 : 0 (2.0 %), C15 : 0 (1.8 %), C16 : 1ω7c (1.7 %), C17 : 1ω7c (0.9 %), C14 : 0 (0.9 %) and C16 : 0 (0.7 %). The chemotaxonomic data are consistent with the assignment of strain NEAU-Jh2-17T to the genus *Streptomonospora*

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Streptomonospora. Its closest relatives were *S. nanhaiensis 12A09T* (99.26 % 16S rRNA gene sequence similarity), *N. rosea YIM 90094T* (97.31 %), *S. halophila YIM 91355T* (97.24 %) and *S. arabica S186T* (97.02 %). The phylogenetic tree based on 16S rRNA gene sequences showed that strain NEAU-Jh2-17T formed a stable clade with *S. nanhaiensis 12A09T* (supported by a high bootstrap value of 100 %; Fig. 2) and the clade adjacent to this group consisted of the type strains of species of the genus *Streptomonospora* in the neighbour-joining tree, while *N. rosea YIM 90094T* together with the two other members of the genus *Nocardiopsis* were separate from strain NEAU-Jh2-17T, although the second highest 16S rRNA gene sequence similarity was found to *N. rosea YIM 90094T*. This relationship was also supported by the maximum-likelihood algorithm (Fig. S2).

DNA–DNA hybridizations were employed to further clarify the relatedness between the novel strain and *S. nanhaiensis 12A09T*, *N. rosea YIM 90094T*, *S. halophila YIM 91355T* and *S. arabica DSM 45083T*. Strain NEAU-Jh2-17T shared mean DNA–DNA relatedness of 30.91 ± 0.70 % with *S. nanhaiensis 12A09T*, 33.35 ± 1.50 % with *N. rosea YIM 90094T*, 43.77 ± 2.73 % with *S. halophila YIM 91355T* and 48.39 ± 1.91 % with *S. arabica DSM 45083T*. These values are below the threshold value of 70 % recommended by Wayne *et al.* (1987) for assigning strains to the same genomic species.

Comparative studies between strain NEAU-Jh2-17T and *S. nanhaiensis 12A09T*, *N. rosea YIM 90094T*, *S. halophila YIM 91355T* and *S. arabica DSM 45083T* revealed that they were significantly different in terms of phenotypic and chemotaxonomic characteristics as summarized in Table 1. For example, strain NEAU-Jh2-17T was unable to grow above 37 °C, in contrast to its closely related species (*S. nanhaiensis 12A09T* and *N. rosea YIM 90094T* were even able to grow at 50 and 60 °C, respectively). Strain NEAU-Jh2-17T was able to tolerate up to 10 % NaCl, a value distinctly lower than those of the four reference strains (18–25 %). In addition, the novel strain was able to utilize L-tyrosine and L-serine but could not hydrolyse aesculin, characteristics that readily distinguish it from its closely related species. Strain NEAU-Jh2-17T could be differentiated from *N. rosea YIM 90094T* based on the

![Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences (1366 bp, omitting unaligned regions) showing the relationship between strain NEAU-Jh2-17T and its closest relatives (including all type strains of species of the genus Streptomonospora). The out-group used was Streptosporangium album DSM 43023T. Only bootstrap values above 50 % (percentages of 1000 replications) are indicated. Asterisks indicate branches also recovered in the maximum-likelihood tree. Bar, 0.01 nt substitutions per site.](image-url)
Table 1. Differential phenotypic and chemotaxonomic characteristics between strain NEAU-Jh2-17<sup>T</sup> and closely related species of the genus *Streptomonospora*

Strains: 1, NEAU-Jh2-17<sup>T</sup>; 2, *S. nanhaiensis* 12A09<sup>T</sup>; 3, *N. rosea* YIM 90094<sup>T</sup>; 4, *S. halophila* YIM 91355<sup>T</sup>; 5, *S. arabica* DSM 45083<sup>T</sup>. Data are from this study except where indicated. Conflicting results from other studies are indicated in parentheses. ND, No data available; +, positive; -, negative.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2*</th>
<th>3*</th>
<th>4*</th>
<th>5*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation of single spores on substrate mycelium</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Single spore surface</td>
<td>Wrinkled</td>
<td>Smooth&lt;sup&gt;a&lt;/sup&gt;</td>
<td>None&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Smooth&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wrinkled&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NaCl concentration for growth (% w/v)</td>
<td>0–10</td>
<td>0–20</td>
<td>0–18</td>
<td>0–25</td>
<td>0–20</td>
</tr>
<tr>
<td>Growth temperature (°C) (optimum)</td>
<td>20–37 (28)</td>
<td>10–50 (37)</td>
<td>20–60 (37–40)</td>
<td>15–45 (37)</td>
<td>10–40 (28)</td>
</tr>
<tr>
<td>Reduction of nitrate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of aesculin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coagulation of milk</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Peptonization of milk</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1-L-Tyrosine</td>
<td>+</td>
<td>-</td>
<td>( + )&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Serine</td>
<td>+</td>
<td>-</td>
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<tr>
<td>L-Alanine</td>
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<tr>
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<td>+</td>
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<td>L-Threonine</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>L-Arginine</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Whole-cell sugars†</td>
<td>Glu, Gal, Rib</td>
<td>Glu, Gal&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Glu, Gal&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Glu, Gal, Rib&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Polar lipids‡</td>
<td>DPG, PG, PC, PE, PL, PIM, PL, GL</td>
<td>DPG, PG, PC, PI, PIM, GL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DPG, PG, PC, PE, PL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DPG, PG, PC, PL, PIM, PL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DPG, PG, PC, PE, PL, PIM, PL, GL&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Predominant menaquinones (&gt;10%)</td>
<td>MK-10(H&lt;sub&gt;2,4,6,8&lt;/sub&gt;)</td>
<td>MK-10(H&lt;sub&gt;4,6&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;, MK-11(H&lt;sub&gt;4,6&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MK-11(H&lt;sub&gt;0,2,4&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;, MK-10(H&lt;sub&gt;6,8&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MK-10(H&lt;sub&gt;4,6,8&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>71.8</td>
<td>74.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data for the reference type strains are from: a, Zhang *et al.* (2013); b, Li *et al.* (2003).

†Glu, Glucose; Gal, galactose; Rib, ribose.
‡DPG, Diphostatidyglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PE, phosphatidylethanolamine; PME, phosphatidymethylethanolamine; PGL, phosphoglycolipid; PL, phospholipid; GL, glycolipid.
formation of single spores on substrate mycelium. Other phenotypic characteristics such as the surface of single spores, reduction of nitrate, coagulation and peptonization of milk, and chemotaxonomic characteristics could also be used to differentiate the new isolate from its closest relatives. Therefore, it is evident from the genotypic and phenotypic data that strain NEAU-Jh2-17T represents a novel species of the genus *Streptomonospora*, for which the name *Streptomonospora halotolerans* sp. nov. is proposed.

**Description of Streptomonospora halotolerans** sp. nov.

*Streptomonospora halotolerans* (ha.lo.to’le.rans. Gr. n. halsalt; L. part. tolerans tolerating; N.L. part. adj. halotolerans referring to the ability to tolerate high salt concentrations).

Gram-positive, aerobic. Good growth on ISP 2, 3, 5 and 7, Czapek’s agar and nutrient agar, moderate growth on ISP 6 agar, but no growth on ISP 4 agar. The aerial mycelium colour is white on ISP 3 and 5 agar. The substrate mycelium colour is in the yellow colour-series. Diffusible pigments are not produced on any of the media tested. At maturity, the aerial mycelium forms sporiferous chains (smooth surfaces) and single spores (wrinkled surfaces) are borne on short sporophores of the substrate mycelium on ISP 3 medium. Aerial and substrate mycelia are not fragmented. Growth occurs at pH 7.0–9.0, the optimum being pH 8.0. Tolerates up to 10.0 % NaCl and grows at temperatures between 20 and 37 °C, with an optimum temperature of 28 °C. Positive for production of H₂S and negative for reduction of nitrate, production of catalase, cellulase and urease, liquefaction of gelatin, hydrolysis of aesculin, starch and Tween 20, 40 and 80, and peptonization of milk. L-arabinose, D-fructose, D-galactose, D-glucose, inositol, lactose, maltose, d-mannose, raffinose, d-mannitol, L-rhamnose, D-ribose, D-sorbitol, sucrose and D-xylene are utilized as sole carbon sources. L-Alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, L-serine, L-threonine and L-tyrosine are utilized as sole nitrogen sources but creatine and glycine are not. Cell wall contains meso-diaminopimelic acid and the whole-cell hydrolysates are glucose, ribose and galactose. The polar lipids contain diphostidyglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinoline (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol and rhamnolipids (RMLs), two unknown phospholipids (PLs) and two unknown glycolipids (GLs). The predominant menaquinones are MK-10(H₂), MK-10(H₄), MK-10(H₆) and MK-10(H₈). Major fatty acids (> 10 %) are C₁₈:₀ 10-methyl, anteiso-C₁₇:₀ C₁₆:₀ 10-methyl, iso-C₁₅:₀ C₁₇:₀ 10-methyl and C₁₈:₀.

The type strain is NEAU-Jh2-17T (=CGMCC 4.7218T = JCM 30347T), isolated from muddy soil collected from a riverbank in Jilin Province, northern China. The DNA G+C content of the type strain is 71.82 mol%.

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**References**


