Streptomyces zhaozhouensis sp. nov., an actinomycete isolated from candelabra aloe (Aloe arborescens Mill)

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A novel endophytic actinomycete, designated strain NEAU-LZS-5T, was isolated from the leaf of candelabra aloe (Aloe arborescens Mill) and characterized using a polyphasic approach. Analysis of the 16S rRNA gene sequence showed that strain NEAU-LZS-5T belongs to the genus Streptomyces and exhibited 99.51 and 97.37 % similarity to Streptomyces sedi YIM 65188T and Streptomyces specialis GW41-1564T, respectively, whereas low similarity values (<97 %) distinguished strain NEAU-LZS-5T from all other species of the genus Streptomyces with validly published names. Two tree-making algorithms also supported the position that strain NEAU-LZS-5T formed a distinct clade with Streptomyces sedi YIM 65188T and Streptomyces specialis GW41-1564T. However, levels of DNA–DNA relatedness between strain NEAU-LZS-5T and Streptomyces sedi YIM 65188T and Streptomyces specialis GW41-1564T were 45.59 and 31.90 %, respectively. A comparative study between strain NEAU-LZS-5T and the type strains of closest related species of the genus Streptomyces revealed that it differed from them in morphological, physiological and biochemical characteristics. Therefore, strain NEAU-LZS-5T represents a novel species of the genus Streptomyces, for which the name Streptomyces zhaozhouensis sp. nov. is proposed. The type strain is NEAU-LZS-5T (=CGMCC 4.7095T =DSM 42101T).

The genus Streptomyces, proposed by Waksman & Henrici (1943), currently encompasses more than 600 species with validly published names. Kim et al. (2003) proposed the emendation of the description of the family Streptomycetaceae, which comprises the genera Streptomyces, Kitasatospora (Zhang et al., 1997) and Streptacidiphilus (Kim et al., 2003). Species of the genus Streptomyces have distinct features, such as Gram-positive cell walls, high DNA G+C contents, the presence of L-L-diaminopimelic acid and absence of characteristic sugars in the cell wall (Locci, 1989; Anderson & Wellington, 2001). During an investigation of potential sources of novel species and novel natural products, strain NEAU-LZS-5T was isolated from the leaf of candelabra aloe (Aloe arborescens Mill). In this study, we performed a polyphasic taxonomic on this strain, and propose that it represents a novel species of the genus Streptomyces.

Strain NEAU-LZS-5T was isolated from the leaf of candelabra aloe collected from Zhaozhou, Heilongjiang Province, northern China (45° 45′ N 126° 41′ E). The plant was tagged outdoors and stored in a clean plastic bag until used (approximately 24 h). The sample was air-dried for 24 h at room temperature and then washed in water with an ultrasonic step (160 W, 15 min) to remove soil and adherent epiphytes. After drying, the sample was cut into pieces 5–10 mm in length and then subjected to a seven-step surface sterilization procedure: a 60 s wash in sterile tap water containing cycloheximide (100 mg l−1) and nalidixic acid (20 mg l−1), followed by a wash in sterile water, a 5 min wash in 5 % (v/v) NaOCl, a 10 min wash in 2.5 % (w/v) Na2S2O3, a 5 min wash in 75 % (v/v)
ethanol, a wash in sterile water and a final rinse in 10% (w/v) NaHCO₃ for 10 min, and then the rinsed sample was dried at 70 °C for 30 min. After being thoroughly dried under sterile conditions, the sample was cut up in a commercial blender and ground with a mortar and pestle, employing 1 ml of 0.5 M potassium phosphate buffer (pH 7.0) per 100 mg tissue. Tissue particles were allowed to settle down at 4 °C for 20–30 min, and the supernatant was spread on a plate of hemic acid-vitamin agar (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). After 14 days of aerobic incubation at 28 °C, colonies were transferred and purified on International Streptomyces Project (ISP) 3 medium (Shirling & Gottlieb, 1966) and incubated at 28 °C for 14–21 days.

Cultural characteristics were determined after 14 days at 28 °C using ISP media 2–7 (Shirling & Gottlieb, 1966), nutrient agar (Waksman, 1961) and Czapek’s agar (Waksman, 1967). The ISCC-NBS colour charts were used to determine the designations of colony colours (Kelly, 1964). Morphological characteristics were observed by light microscopy (Nikon ECLIPSE E200) and electron microscopy (Hitachi S-3400N) using cultures grown on ISP3 agar at 28 °C for 14 days. The utilization of sole carbon and nitrogen, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, peptonization of milk, liquefaction of gelatin and production of H₂S were examined as described previously (Gordon et al., 1974; Yokota et al., 1993).

Production of catalase, esterase and urease were tested as described by Smibert & Krieg (1994). Growth at different temperatures (4, 16, 18, 22, 28, 32, 35, 37 °C) was determined on ISP3 medium after incubation for 14 days. Growth tests for pH range were carried out by using media adjusted to pH 2–12 with 4 M HCl or 5 M KOH after sterilization, and NaCl tolerance was determined in GY medium [1.0 % (w/v) yeast extract, 1.0 % glucose, 0.05 % K₂HPO₄ and 0.05 % MgSO₄, pH 7.2] supplemented with 0–7 % NaCl (w/v) at 28 °C for 14 days on a rotary shaker.

Biomass for chemical studies was prepared by growing the strain in GY medium in Erlenmeyer flasks for 7 days at 28 °C. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomer of diaminopimelic acid in whole-cell hydrolysates was derivatized according to McKerrow et al. (2000) and analysed by HPLC using an Agilent TC-C₁₈ column (250 × 4.6 mm, inner diameter 5 μm) with a mobile phase consisting of acetonitrile/0.05 mol phosphate buffer (pH 7.2) 1⁻¹ (15 : 85) at a flow rate of 0.5 ml min⁻¹. Peak detection used an Agilent G1321A fluorescence detector with a 365 nm excitation and a 455 nm longpass emission filter.

Whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). The phospholipids 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 with an HPLC-UV method using an Agilent Extend-C₁₈ column (150 × 4.6 mm, inner diameter 5 μm), typically at 270 nm. The mobile phase was acetonitrile/propyl alcohol (60 : 40, v/v) and the flow rate was set to 1.0 ml min⁻¹ and the run time was set to 60 min. The injection volume was 20 μl, and the chromatographic column was controlled at 40 °C (Wu et al., 1989). Mycolic acids were checked by the acid methanolysis method as described by Minnikin et al. (1980). Fatty acids were analysed by GC-MS using the method of Xiang et al. (2011).

Extraction of chromosomal DNA and PCR-mediated amplification of the 16S rRNA gene were 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) and software provided by the manufacturer. The almost full-length 16S rRNA gene sequence of strain NEAU-LZS-5ᵀ was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X 1.83 software. Phylogenetic trees were generated with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA version 5.05 (Tamura et al., 2011). The stability of the clad in the trees was appraised using a bootstrap value with 1000 repeats (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

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### Table 1. Growth and cultural characteristics of strain NEAU-LZS-5ᵀ

<table>
<thead>
<tr>
<th>Agar medium</th>
<th>Growth</th>
<th>Aerial mycelium</th>
<th>Substrate mycelium</th>
<th>Diffusible pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract/malt extract (ISP2)</td>
<td>Good</td>
<td>White</td>
<td>White</td>
<td>None</td>
</tr>
<tr>
<td>Oatmeal (ISP3)</td>
<td>Good</td>
<td>White</td>
<td>Vivid yellowish green</td>
<td>None</td>
</tr>
<tr>
<td>Inorganic salts/starch (ISP4)</td>
<td>Poor</td>
<td>None</td>
<td>White</td>
<td>None</td>
</tr>
<tr>
<td>Glycerol/asparagine (ISP5)</td>
<td>Good</td>
<td>White</td>
<td>Light Yellow Green</td>
<td>None</td>
</tr>
<tr>
<td>Peptone/yeast extract/iron (ISP6)</td>
<td>Poor</td>
<td>Pale yellowish pink</td>
<td>Yellow</td>
<td>None</td>
</tr>
<tr>
<td>Tyrosine (ISP7)</td>
<td>Good</td>
<td>White</td>
<td>Yellow</td>
<td>None</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Good</td>
<td>Pale yellow</td>
<td>Pale yellowish</td>
<td>None</td>
</tr>
<tr>
<td>Czapek’s</td>
<td>Poor</td>
<td>None</td>
<td>Yellowish white</td>
<td>None</td>
</tr>
</tbody>
</table>
strains were calculated on the basis of pairwise alignment using the EzTaxon-e server (Kim et al., 2012). The G+C content of the genomic DNA was determined using the thermal denaturation ($T_m$) method (Mandel & Marmur, 1968) with *Escherichia coli* JM109 as the control. DNA–DNA relatedness tests between strain NEAU-LZS-5$^T$ and *Streptomyces sedi* YIM 65188$^T$ and *Streptomyces specialis* GW41-1564$^T$ 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 multicell changer and a temperature controller with *in-situ* temperature probe (Varian).

Morphological observation of a 14 day culture of strain NEAU-LZS-5$^T$ grown on ISP3 agar revealed that aerial mycelium was abundant and substrate mycelium was well developed without fragmentation. Non-motile spores (0.6–2.0 × 0.7–2.8 μm) were borne singly on the aerial mycelium and the spore surface was smooth (Fig. S1, available in the online Supplementary Material). Sporangia were not found. Cultural characteristics of strain NEAU-LZS-5$^T$ are detailed in Table 1. Strain NEAU-LZS-5$^T$ grew well on ISP2, ISP3, ISP5, ISP7 and nutrient agar. Poor growth was observed on ISP4, ISP6 and Czapek's agar. Diffusible pigments were not formed on any tested media. Growth of strain NEAU-LZS-5$^T$ occurred at pH 6–10 and with 0–7%
(w/v) NaCl, with optimum growth at pH 7.0 and with 1 % (w/v) NaCl. The temperature range for growth was 16–35 °C, with the optimum temperature being 28 °C. Detailed physiological characteristics are presented in the species description.

The cell wall of strain NEAU-LZS-5^T contained L-l-diaminopimelic acid and glycline, indicating that it has a cell wall of chemotype I (Lechevalier & Lechevalier, 1970a, b). The whole-cell hydrolysate contained glucose. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and three unknown phospholipids (phospholipid type II sensu Lechevalier et al., 1977) (Fig. S2). The predominant menaquinones were MK-9(H_4) (16.7 %), MK-9(H_6) (29.3 %), MK-9(H_8) (43.4 %) and MK-10(H_6) (10.6 %). The major fatty acids were C_{16:0} (35.48 %), C_{17:1}ω7c (12.97 %), C_{15:0}ω6c (9.98 %), iso-C_{15:0} (7.80 %), C_{18:0}ω7c (7.64 %), C_{16:1}ω7c (7.49 %) and anteiso-C_{17:0} (7.24 %). Mycolic acids were not detected.

Sequence analysis of the 16S rRNA gene sequence showed that strain NEAU-LZS-5^T was affiliated to the genus Streptomyces. Based on EzTaxon-e analysis, strain NEAU-LZS-5^T was related most closely to Streptomyces sedi YIM 65188^T and Streptomyces specialis GW41-1564^T (99.51 and 97.37 % 16S rRNA gene sequence similarity, respectively). 16S rRNA gene sequence similarities between the new isolate and other type strains of species of the genus Streptomyces were lower than 97 %. The phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences showed that strain NEAU-LZS-5^T formed a distinct branch with Streptomyces sedi YIM 65188^T and Streptomyces specialis GW41-1564^T that was supported by a bootstrap value of 55 % in the neighbour-joining tree and also recovered with the maximum-likelihood algorithm (Fig. S3). However, DNA–DNA hybridization experiments revealed mean levels of relatedness of 45.59 ± 1.89 and 31.9 ± 1.54 % relatedness between strain NEAU-LZS-5^T and Streptomyces sedi YIM 65188^T and Streptomyces specialis GW41-1564^T, respectively, which are well below the 70 % value that is considered to be the threshold for the delineation of genomic species (Wayne et al., 1987).

According to Kim et al. (2003), members of the genus Streptomyces possess spores arranged in chains. However, spores of strain NEAU-LZS-5^T occurred singly. To verify this result, an analysis of strain NEAU-LZS-5^T grown independently on YIM 38 medium (Li et al., 2009) and DSMZ medium 65 (Kämpfer et al., 2008) was carried out. The result showed that strain NEAU-LZS-5^T produced single spores on both media. Spores of Streptomyces sedi YIM 65188^T grown on ISP medium 3 under the same conditions appeared as chains (Fig. S1). In addition, the isolate could also be clearly distinguished from Streptomyces sedi YIM 65188^T and Streptomyces specialis GW41-1564^T based on physiological and biochemical characteristics, as summarized in Table 2. Furthermore, the predominant menaquinones of strain NEAU-LZS-5^T are MK-9(H_4) and MK-9(H_8), which are significantly different from those of

**Streptomyces zhaozhouensis sp. nov.**

Streptomyces zhaozhouensis (zhao.zhou.en’sis. N.L. masc. adj. zhaozhouensis pertaining to Zhaozhou, Heilongjiang Province, China, where the type strain was isolated).

Aerobic, Gram-staining positive actinomycete that forms an extensively branched substrate mycelium and aerial hyphae that differentiate into single spores (0.60–2.0 × 0.70–2.80 μm) that are elliptical. Grows well on ISP2, ISP3, ISP5, ISP7 and nutrient agar; poor growth is observed on ISP4, ISP6 and Czapek’s agar. Diffusible pigments are not formed on any tested media. Utilizes D-galactose, D-glucose, lactose, D-mannitol, maltose, sucrose and D-xylose as sole carbon sources but not L-arabinose, D-fructose, inositol, D-mannose, raffinose, L-rhamnose, D-ribose or D-sorbitol. L-Alanine, L-arginine, L-aspartic acid, L-asparagine, creatine, L-glutamic acid, L-serine, L-threonine and L-tyrosine can be used as sole nitrogen sources but not L-glutamine or glycine. Growth occurs at pH 6–10 and with 0–7 % (w/v) NaCl, with optimum growth at pH 7.0 and with 1 % (w/v) NaCl. The temperature range for growth is 16–35 °C, with

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Spore arrangement</td>
<td>Single</td>
<td>None</td>
<td>Spirals</td>
</tr>
<tr>
<td>Diffusible pigment</td>
<td>(on ISP3 medium)</td>
<td>Dark green</td>
<td>Black</td>
</tr>
<tr>
<td>Liquefaction of gelatin</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Decomposition of urea</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Decomposition of cellulose</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of aesculin</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Arginine</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
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the optimum temperature being 28 °C. Positive for production of catalase, liquefaction of gelatin and hydrolysis of aesculin, but negative for decomposition of cellulose and urea, production of H₂S and oxidase, hydrolysis of starch and Tween 80, and reduction of nitrate. The cell wall is of chemotype I and the whole-cell sugar is glucose. The major menaquinones are MK-9(H₆) and MK-9(H₈). The phospholipid profile comprises phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerolphosphate, and three unknown phospholipids. The major fatty acids are C₁₆:0, C₁₇:1ω7c, C₁₅:0, iso-C₁₅:0, C₁₈:0, C₁₆:1ω7c and anteiso-C₁₇:0.

The strain type is NEAU-LZS-5T (=CGMCC 4.7095T = DSM 42101T), isolated from the leaf of candelabra aloe (Aloe arborescens Mill) collected from Zhaozhou, Heilongjiang Province, northern China. The DNA G+C content of the type strain is 67.80 ± 0.25 mol%.

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References


