Streptacidiphilus monticola sp. nov., a novel actinomycete isolated from soil

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Abstract

A novel actinobacterium, designated strain NEAU-SW11T, was isolated from soil collected from Binxian, Heilongjiang province, north China. The isolate was found to have chemical and morphological properties of the genus Streptacidiphilus, with the highest sequence similarities to Streptacidiphilus amyonensis JCM 16223T (98.1 %), Streptacidiphilus jiangxiensis JCM 12277T (97.8 %), Streptacidiphilus melanogenes JCM 16224T (97.6 %) and Streptacidiphilus rugosus JCM 16225T (97.4 %) and it phylogenetically clustered with these four strains. The cell wall contained L,L-diaminopimelic acid as the major diamino acid and the whole-cell hydrolysates were rhamnose, ribose, glucose and galactose. The major polar lipids were diphasophatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and two unidentified phospholipids. The predominant menaquinones were MK-9(H6) and MK-9(H8). The major fatty acids were C16:0, anteiso-C17:0, C14:0 and C15:0. The DNA G+C content was 71.0 mol%. However, DNA–DNA hybridization, physiological and biochemical data showed that strain NEAU-SW11T could be distinguished from its closest relatives. Therefore, strain NEAU-SW11T represents a novel species of the genus Streptacidiphilus, for which the name Streptacidiphilus monticola sp. nov. is proposed. The type strain is NEAU-SW11T (=CGMCC 4.7427=DSM 105744T).

The genus Streptacidiphilus, belonging to the family Streptomycetaceae, was established by Kim et al. [1]. At the time of writing, 11 species of Streptacidiphilus are described, including the latest described species Streptacidiphilus torenensis [2]. Members of the genus form extensively branched substrate mycelia and aerial hyphae that differentiate into long chains of smooth-surfaced spores [3]. Members of the genus Streptacidiphilus are common and widely distributed in acidic habitats, notably in coniferous soils [4], are a source of antifungal compounds [5] and are implicated in the turnover of organic matter at low pH values [6,7]. In the course of an investigation of rare actinobacteria, strain NEAU-SW11T was isolated. Here we report on the taxonomic characterization and classification of the isolate and propose that strain NEAU-SW11T represents a new species of the genus Streptacidiphilus.

Strain NEAU-SW11T was isolated from soil collected at 10–15 cm depth at Xianglu Mountain, Binxian, Heilongjiang Province, north China (45°75’ N, 127°48’ E). The soil is cloddy Baijiang soil and is grey in colour. The soil texture ranges from medium loam to heavy loam, and the pH value is 6.2. The sample was processed as described by Zheng et al. [8] and placed on a plate of cellulose–proline agar [9] supplemented with cycloheximide (50 mg l–1) and nalidixic acid (50 mg l–1), the medium pH was 7.2. After 15 days of aerobic incubation at 28°C, colonies were transferred and purified on oatmeal agar [International Streptomyces Project (ISP) 3 medium] [10] and maintained as glycerol suspensions (20%, v/v) at –80°C.

Morphological characteristics were observed by light microscopy (Nikon Eclipse E200) and scanning electron microscopy (Hitachi SU8010) using cultures grown on ISP 3 agar at 28°C for 4 weeks. Cultural characteristics were determined on ISP media 1–7 [10], Czapek’s agar [11], Bennett’s agar [12] and nutrient agar [13] after 14 days at 28°C. The colour of strain NEAU-SW11T was determined with
colour chips from the ISCC-NBS colour charts [14]. Growth at different temperatures (10, 15, 20, 25, 28, 35, 37, 40 and 45 °C) was determined on ISP 3 medium after incubation for 14 days. Growth tests for pH range (pH 4.0–11.0, at intervals of 1.0 pH unit) [15] and NaCl tolerance (0, 1, 2, 3, 4, 5, 6, and 7%, w/v) were tested on ISP 2 medium at 28 °C for 14 days on a rotary shaker. Hydrolysis of Tweens (20, 40 and 80) and production of urease were tested as described by Smibert and Krieg [16]. The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, coagulation and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, coagulation and peptonization of milk, liquefaction of gelatin and production of H₂S were examined as described previously [17, 18].

Biomass for chemotaxonomic analysis was prepared by growing the novel strain in ISP 2 broth in shake flasks at 28 °C for 7 days, washed with distilled water twice and freeze-dried. The isomer of diaminopimelic acid (DAP) in the cell-wall hydrolysates was derivatised and analysed by a high-performance liquid chromatography (HPLC) method [19]. The whole-cell sugars were analysed according to the procedures developed by Lechevalier and Lechevalier [20]. The polar lipids were examined by two-dimensional thin-layer chromatography and identified using the method of Minnikin et al. [21]. Menaquinones were extracted from freeze-dried biomass and purified according to Collins [22]. Extracts were analysed by a HPLC-UV method [23] using an Agilent Extend-C₁₈ column (150 × 4.6 mm, i.d. 5 μm) at 270 nm. The mobile phase was acetonitrile:iso-propyl alcohol (60:40, v/v). To determine cellular fatty acid compositions, strain NEAU-SW11ᵀ was cultivated in ISP 2 broth in shake flasks at 28 °C for 7 days. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. [24] and analysed by gas chromatography–mass spectrometry using the method of Xiang et al. [25] and identified with the NIST MS Search 2.0 database.

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence were carried out according to the procedure developed by Kim et al. [26]. The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). An almost full-length 16S rRNA gene sequence of strain NEAU-SW11ᵀ (1509 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL_X 1.83 software. Phylogenetic trees were reconstructed with the neighbour-joining [27] and maximum likelihood [28] algorithms using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.06 [29]. The stability of the topology of the phylogenetic tree was assessed using the bootstrap method with 1000 repetitions [30]. A distance matrix was generated using Kimura’s two-parameter model [31]. 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 Ezbiocloud server [32].

The G+C content of the genomic DNA was determined using the thermal denaturation (Tₘ) method [33] with Escherichia coli JM109 DNA used as the control. DNA–DNA relatedness tests between the isolate NEAU-SW11ᵀ and related type strains Streptacidiphilus anmyonensis JCM 16223ᵀ, Streptacidiphilus jiangxiensis JCM 12277ᵀ, Streptacidiphilus malanogenes JCM 16224ᵀ, and Streptacidiphilus rugosus JCM 16225ᵀ were carried out as described by De Ley et al. [34] under consideration of the modifications described by Huss et al. [35], using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with an in situ temperature probe (Varian). The concentration and purity of DNA samples were determined by measuring the absorbance at 260, 280 and 230 nm. The DNA samples used for hybridization were diluted to OD₂₆₀ around 1.0 using 0.1× SSC (sodium citrate buffer [36]), then sheared using a JY92-II ultrasonic cell disruptor (ultrasonic time 3 s, interval time 4 s, 90 times). The DNA renaturation rates were determined in 2× SSC at 70 °C. The experiments were performed with three

![Fig. 1. Scanning electron micrographs of strain NEAU-SW11ᵀ grown on ISP 3 agar for 4 weeks at 28 °C.](image)

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replications and the DNA–DNA relatedness value was expressed as a mean.

Morphological observation of a 4-week culture of strain NEAU-SW11T grown on ISP 3 medium revealed it shared the same morphological characteristics as members of the genus *Streptacidiphilus*. The isolate formed well-developed substrate mycelia and aerial mycelia that differentiated into flexuous to straight spore chains consisting of cylindrical spores (0.8–0.9×1.0–1.1 μm) (Fig. 1a). The surfaces of the spores were covered with sheath-like structures, the sheath surface was rough, but the spore surface was smooth (Fig. 1b). Strain NEAU-SW11T exhibited good growth on ISP 2, 3, 5 and 7, and Bennett’s agar, moderate growth on ISP 1 and Czapek’s agar, poor growth on nutrient agar, but no growth on ISP 4, ISP 6 or TSA media. No diffusible pigments were observed on any of the media tested for the isolate. Strain NEAU-SW11T grew well between pH 5.0 and 9.0, with an optimum pH of 6.0. The range of temperature was determined to be 15–40 °C, with the optimum growth temperature being 28 °C. Strain NEAU-SW11T grew in the presence of 0–2 % NaCl (w/v), with the optimum condition at 0 %. Detailed physiological characteristics are presented in the species description.

Chemotaxonomic analyses revealed that strain NEAU-SW11T exhibited characteristics typical of members of the genus *Streptacidiphilus*. Strain NEAU-SW11T was found to contain LL-diaminopimelic acid as the major diamino acid (76 %) and a minor amount of meso-diaminopimelic acid (24 %) (Fig. S1, available in the online version of this article). The whole-cell hydrolysates were determined to contain rhamnose, ribose, glucose and galactose. The polar lipids were diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphorylated lipids and the DNA–DNA relatedness value was expressed as a mean.

Based on Ezbiocloud analysis, strain NEAU-SW11T should be assigned to the genus *Streptacidiphilus*, with the highest 16S rRNA gene sequence similarity to *S. anmyonensis* JCM 16223T (98.1 %). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain NEAU-SW11T formed a stable clade with *S. anmyonensis* JCM 16223T (98.1 %), *S. jiangxiensis* JCM 12277T (97.8 %), *S. melanogenes* JCM 16224T (97.6 %) and *S. rugosus* JCM 16225T (97.4 %), which was supported by a high bootstrap value of 85 % (Fig. 2). This relationship was also observed in the maximum-likelihood tree (Fig. S3). DNA–DNA hybridization was employed to further distinguish strain NEAU-SW11T from *S. anmyonensis* JCM 16223T, *S. jiangxiensis* JCM 12277T, *S. melanogenes* JCM 16224T and *S. rugosus* JCM 16225T. The levels of DNA–DNA relatedness between them were 51.2±4.2, 45.5±4.9, 42.3±5.6 and 40.4±3.7 %, respectively. These values are below the threshold value of 70 % recommended by Wayne et al. [37] for assigning strains to the same genomic species.

Besides the genotypic evidence above, strain NEAU-SW11T can also be distinguished from its closely related strains by several phenotypic characteristics (Table S1) such as the different colony colours on ISP 2, ISP 3, ISP 5, ISP 7 and Bennett’s media (Fig. S4). The strain could grow at 40 °C, in contrast to its closely related strains. Other phenotypic
Table 1. Differential characteristics of strain NEAU-SW11\textsuperscript{T} and closely related species of the genus Streptacidiphilus

<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>Coagulation and peptonization of milk</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Decomposition of cellulose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>Hydrolysis of aesculin</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Degradation of Tween 40</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Degradation of Tween 80</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization as sole carbon source:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>d-Sorbitol</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>D-Xylose</td>
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<td>+</td>
<td>−</td>
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<td>+</td>
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<tr>
<td>Utilization as sole nitrogen source:</td>
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<tr>
<td>Creatine</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glutamate</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>4–9</td>
<td>3–8\textsuperscript{a}</td>
<td>3–8</td>
<td>3–8\textsuperscript{a}</td>
<td>3–8\textsuperscript{a}</td>
</tr>
<tr>
<td>Optimal pH for growth</td>
<td>6.0</td>
<td>5.0</td>
<td>5.0</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>NaCl concentration for growth (%, w/v)</td>
<td>0–2</td>
<td>0–2</td>
<td>0–3</td>
<td>0–3</td>
<td>0–4</td>
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<tr>
<td>Optimal NaCl for growth (%, w/v)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Growth temperature (\°C)</td>
<td>15–40</td>
<td>28–35\textsuperscript{a}</td>
<td>15–37</td>
<td>28–35\textsuperscript{a}</td>
<td>28–35\textsuperscript{a}</td>
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<tr>
<td>Optional temperature for growth (\°C)</td>
<td>28</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>DNA G+C content (%)</td>
<td>71.0</td>
<td>70.1</td>
<td>70.6</td>
<td>71.4</td>
<td>71.3</td>
</tr>
<tr>
<td>Ll-DAP (%)</td>
<td>76</td>
<td>89\textsuperscript{a}</td>
<td>80</td>
<td>100\textsuperscript{a}</td>
<td>94\textsuperscript{a}</td>
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<tr>
<td>Meso-DAP(%)</td>
<td>24</td>
<td>11\textsuperscript{a}</td>
<td>20</td>
<td>0\textsuperscript{a}</td>
<td>6\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Data from Cho SH et al. [3].

differences, including coagulation and peptonization of milk, hydrolysis of aesculin, decomposition of cellulose, degradation of Tween 40 and 80, and utilization of d-sorbitol and myo-inositol, are summarized in Table 1. Therefore, it is evident from the genotypic and phenotypic data that strain NEAU-SW11\textsuperscript{T} represents a novel species of the genus Streptacidiphilus, for which the name Streptacidiphilus monticola sp. nov. is proposed.

**DESCRIPTION OF STREPTACIDIPHILUS MONTICOLA SP. NOV.**

*Streptacidiphilus monticola* (mon.ti’co.la. L. masc. n. monti-cola mountain-dweller).

Gram-stain-positive, aerobic actinobacterium that forms well-developed substrate mycelia and aerial mycelia that differentiate into flexuous to straight spore chains consisting of cylindrical spores (0.8–0.9×1.0–1.1 \textmu m) (Fig. 1a). The spore surface is smooth. Good growth on ISP 2, ISP 3, ISP 5, ISP 7 and Bennett’s media; moderate growth on ISP 1 and Czapek’s media; poor growth on nutrient agar; no growth on ISP 4, ISP 6 or trypticase soy agar media. No diffusible pigments are observed on any of the media tested. Growth occurs at pH values between 5.0 and 9.0, the optimum being pH 6.0. Tolerates up to 2.0 % NaCl, with the optimum condition at 0 %. Grows at temperatures between 15 and 40 °C, with an optimum temperature of 28 °C. Positive for hydrolysis of starch, production of cellulase and coagulation and peptonization of milk, and negative for production of H\textsubscript{2}S and urease, hydrolysis of aesculin, liquefaction of gelatin, reduction of nitrate and decomposition of Tweens (20, 40 and 80). D-Fructose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, and sucrose are utilized as sole carbon sources, but not L-arabinose, D-inositol, D-ribose, D-sorbitol or D-xylose. L-Alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, glycine, L-proline, L-serine, L-threonine and L-tyrosine are utilized as sole nitrogen sources, but not creatine. Strain NEAU-SW11\textsuperscript{T} is found to contain Ll-diaminopimelic acid as a major diamino acid (76 %) and a minor amount of meso-diaminopimelic (24 %). The whole-cell hydrolysates are rhamnose, ribose, glucose and galactose. The polar lipids contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and two unidentified phospholipids. The predominant menaquinones are MK-9(H\textsubscript{4}) and MK-9(H\textsubscript{6}). Major fatty acids (>10 %) are C\textsubscript{16}-0, anteiso-C\textsubscript{17}-0, C\textsubscript{14}-0 and C\textsubscript{15}-0. The DNA G+C content of the type strain is 71.0 mol%.

The type strain is NEAU-SW11\textsuperscript{T} (=CGMCC 4.7427\textsuperscript{T}=DSM 105744\textsuperscript{T}), isolated from soil collected from Xianglu Mountain, Binxin, Heilongjiang Province, north China.
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Conflicts of interest
The authors declare that there are no conflicts of interest.

References