**Streptomyces tritici** sp. nov., a novel actinomycete isolated from rhizosphere soil of wheat (*Triticum aestivum* L.)

Junwei Zhao,† Linlin Shi,† Wenchao Li,† Jiabin Wang,† Han Wang,† Yuanyuan Tian,† Wensheng Xiang‡,* and Xiangjing Wang†,*

**Abstract**

Two novel actinomycete isolates, designated strains NEAU-A4⁷ and NEAU-A3, were isolated from rhizosphere soil of wheat (*Triticum aestivum* L.) and characterized using a polyphasic approach. Morphological and chemotaxonomic characteristics of the two strains coincided with those of the genus *Streptomyces*. The 16S rRNA gene sequence analysis showed that the two isolates exhibited 99.6 % 16S rRNA gene sequence similarity with each other and that they were most closely related to *Streptomyces violaceorec tusc* DSM 40279⁷ (98.8, 99.0 %). Phylogenetic analysis based on 16S rRNA gene sequences indicated that the two strains clustered together and formed a separate subclade. Furthermore, a combination of DNA–DNA hybridization results and some physiological and biochemical properties demonstrated that the two strains could be distinguished from its closest relative. Therefore, it is proposed that strains NEAU-A4⁷ and NEAU-A3 should be classified as representatives of a novel species of the genus *Streptomyces*, for which the name *Streptomyces tritici* sp. nov. is proposed. The type strain is NEAU-A4⁷ (=CGMCC 4.7393⁷=DSM 104540⁷).

The genus *Streptomyces* within the family *Streptomycetaceae*, which was firstly proposed by Waksman and Henrici [1], currently encompasses more than 800 species with validly published names ([www.bacterio.net/streptomyces.html](http://www.bacterio.net/streptomyces.html)). As the largest genus of the phylum *Actinobacteria*, *Streptomyces* are widely distributed in soils throughout the world and have a wide range of metabolic abilities and potential applications in the production of antibiotics, enzymes, enzyme inhibitors, vitamins and bioactive compounds with importance in the food, agriculture and pharmaceutical industries [2, 3].

Microbes associated with plant roots are crucial for plant health and referred to as the second genome of the plant. When a plant is attacked by plant-pathogenic microorganisms, the diversity and community structure of plant-associated microbes might be changed and the plant can recruit and activate plant-beneficial microorganisms [4, 5]. During an investigation of the diversity and community structure and function of microbes associated with wheat attacked by *Puccinia striiformis* f. sp. *tritici*, two aerobic actinomycetes, NEAU-A4⁷ and NEAU-A3, were isolated from rhizosphere soil of wheat and subjected to the polyphasic taxonomy analysis. The results demonstrated that they represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces tritici* sp. nov. is proposed.

Strains NEAU-A4⁷ and NEAU-A3 were isolated from rhizosphere soil of wheat (*Triticum aestivum* L.) collected from Langfang, Hebei Province, Central China (39° 32′ N, 116° 40′ E). The rhizosphere soil sample was ground into powder and then suspended in sterile distilled water followed by a standard serial dilution technique. The diluted soil suspension was spread on humic acid–vitamin agar [6] supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). After 28 days of aerobic incubation at 28 °C, colonies were transferred and purified on International *Streptomyces* Project (ISP) medium 3 [7] and maintained as glycerol suspensions (20 %, v/v) at −80 °C.

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**Keywords:** *Streptomyces tritici* sp. nov.; polyphasic taxonomy; 16S rRNA gene.

**Abbreviations:** CGMCC, China General Microbiological Culture Collection Center; CSCP, Chang Jiang Scholar Candidates Program; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; GY, glucose-yeast extract powder; ISCC-NBS, Inter-Society Color Council – National Bureau of Standards; ISP, International *Streptomyces* Project; MEGA, Molecular Evolutionary Genetics Analysis; SSC, saline-sodium citrate.

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains NEAU-A4⁷ and NEAU-A3 are KY744943 and KY744942, respectively.

One supplementary table and three supplementary figures are available with the online version of this article.

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Gram-staining was carried out by using the standard Gram stain and 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 3 weeks. Cultural characteristics were determined on the ISP media 2–7 [7], Czapek’s agar [8], Bennett’s agar [9] nutrient agar [10] and tryptic soy agar (tryptone 15 g; soy peptone 5 g; NaCl 5 g; agar 15 g; distilled water, 1 l, pH 7.2) after 14 days at 28 °C. Colour determination was done with colour chips from the ISCC–NBS colour charts [11]. Growth at different temperatures (10, 15, 20, 25, 28, 32, 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) and NaCl tolerance (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 %, w/v) were tested in glucose-yeast extract powder (GY) medium [12] 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 [13]. The utilization of sole carbon 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 [14, 15].

Biomass for chemical studies was prepared by growing the organisms in GY medium in shake flasks at 28 °C for 4 days. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomer of diaminopimelic acid in the cell-wall hydrolysates was derivatised and analysed by high-performance liquid chromatography (HPLC) method [16] using an Agilent TC-C18 column (250 × 4.6 mm i.d. 5 µm) with a mobile phase consisting of acetonitrile–iso-propyl alcohol (60 : 40, v/v) at 0.05 mol l⁻¹ phosphate buffer pH 7.2 (15 : 85, v/v) at a flow rate of 0.5 ml min⁻¹. The peak detection used an Agilent G1321A fluorescence detector with 365 nm excitation and 455 nm longpass emission filters. The whole-cell sugars were determined in glucose-yeast extract powder (GY) medium [12] at 28 °C after incubation for 14 days. Fatty acid methyl esters were extracted from the biomass by using the method of Minnikin et al. [18]. Menaquinones were extracted from freeze-dried biomass and purified according to Collins [19]. Extracts were analysed by an HPLC-UV method [20] 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, strains NEAU-A4T, NEAU-A3 and Streptomyces violaceoruber DSM 40279T were cultivated in GY medium in shake flasks at 28 °C for 4 days. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. [21] and analysed by gas chromatography–mass spectrometry using the method of Xiang et al. [22] 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 using a standard procedure [23]. The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). Almost full-length 16S rRNA gene sequences of strains NEAU-A4T and NEAU-A3 were obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDJB databases using CLUSTAL_X 1.83 software. Phylogenetic trees were reconstructed with neighbour-joining [24] and maximum likelihood [25] algorithms using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.06 [26]. The stability of the topology of the phylogenetic tree was assessed using the bootstrap method with 1000 repetitions [27]. A distance matrix was generated using Kimura’s two-parameter model [28]. 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 [29].

The G+C contents of the genomic DNA were determined using the thermal denaturation (Tm) method [30] with Escherichia coli JM109 DNA used as the control. DNA–DNA relatedness tests between the two novel isolates and their most closely related type strain S. violaceoruber DSM 40279T were carried out as described by De Ley et al. [31] under consideration of the modifications described by Huss et al. [32], using a 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 optical density at 260, 280 and 230 nm. The DNA samples used for hybridization were diluted to OD260 around 1.0 using 0.1 × SSC (saline sodium citrate buffer), 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 replicates and the DNA–DNA relatedness value was expressed as mean of the three values.

The morphological characteristics of strains NEAU-A4T and NEAU-A3 showed that the two strains had the typical characteristics of the genus Streptomyces. Observation of 3 weeks cultures of strains NEAU-A4T and NEAU-A3 grown on ISP 3 medium revealed that they formed well-developed, branched substrate hyphae and aerial mycelium that differentiated into straight or flexuous spore chains consisted of cylindrical spores (0.6–0.9 × 1.0–1.9 µm) (Fig. 1a, c). The spore surface of strain NEAU-A4T was wrinkled (Fig. 1b), that of strain NEAU-A3 was found to be rough (Fig. 1d). Both strains exhibited good growth on ISP 1, ISP 2, ISP 3, ISP 4, ISP 6, Czapek’s, Bennett’s, nutrient agar and tryptic soy agar, moderate growth on ISP 5 agar, and poor growth on ISP 7 agar. Greenish black diffusible pigment was observed on ISP 4 agar of strain NEAU-A4T. Cultural characteristics of these two strains are shown in Table S1 (available in the online version of this article). The two isolates grew well between pH 6.0 and 11.0, with an optimum pH of 7.0. The range of temperature of these two
strains was determined to be 18–40 ºC, with the optimum growth temperature being 28 ºC. Both strains grew in the presence of 0–5 % NaCl (w/v) and optimally at 0–1 % (w/v). Detailed physiological characteristics are presented in the species description.

Strains NEAU-A4T and NEAU-A3 were found to contain LL-diaminopimelic acid as the diamino acid. The whole-cell hydrolysates of these two strains were determined to contain ribose and glucose. The menaquinones of strains NEAU-A4T and NEAU-A3 were MK-9(H8) (68.1, 61.6 %), MK-9(H6) (18.1, 26.6 %) and MK-10(H2) (13.8, 11.8 %). The cellular fatty acid profiles of strains NEAU-A4T and NEAU-A3 were composed of iso-C16:0 (25.6, 29.9 %), anteiso-C15:0 (22.7, 29.3 %), C16:0 (17.1, 23.8 %), anteiso-C17:0 (13.8, 6.4 %), iso-C15:0 (9.4, 7.9 %), C18:0 (6.8, 0 %) and C17:0 (4.6, 2.7 %). The polar lipids of strain NEAU-A4T consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol mannoside and one unidentified lipid (Fig. S1). All the chemotaxonomic data are consistent with the assignment of strains NEAU-A4T and NEAU-A3 to the genus Streptomyces.

Sequence analysis of the 16S rRNA gene showed that strains NEAU-A4T and NEAU-A3 were affiliated to the genus Streptomyces and exhibited 99.6 % 16S rRNA gene sequence similarity with each other. Based on EzBioCloud analysis, strains NEAU-A4T and NEAU-A3 were most closely related to S. violaceorectus DSM 40279T (98.8, 99.0 %), Streptomyces bikiniensis NRRL B-1049T (98.5, 98.8 %), Streptomyces tanshiensis LMG 20274T (98.5, 98.8 %) and Streptomyces nashvillensis NBRC 13064T (98.5, 98.8 %). The phylogenetic tree based on 16S rRNA gene sequence showed that the two strains clustered together with a bootstrap value of 100 % and formed a separate subclade in the neighbour-joining tree (Fig. 2), a relationship also recovered by the maximum-likelihood algorithm (Fig. S2). DNA–DNA hybridization was employed to further clarify the relatedness between the two strains and S. violaceorectus DSM 40279T. Results showed that strains NEAU-A4T/NEAU-A3 shared DNA–DNA relatedness of 48.1±3.4/47.5±5.2 with S. violaceorectus DSM 40279T. These two values are below the threshold value of 70 % recommended by Wayne et al. [33] for assigning strains to the same genomic species. The DNA relatedness of the two isolates was 81.3±4.5 %, indicating that they belong to the same genomospecies. The DNA G+C contents of strains NEAU-A4T and NEAU-A3 were 71.9±0.2 and 71.5±0.5 mol%, respectively.

Comparison of phenotypic characteristics between strains NEAU-A4T/NEAU-A3 and its closely related species, S. violaceorectus DSM 40279T, was performed to differentiate the strains (Tables 1 and S1, Fig. S3). Differential cultural characteristics included: strain NEAU-A4T could produce greenish black diffusible pigment on ISP 4 medium, in contrast with S. violaceorectus DSM 40279T where no diffusible pigment was observed on this medium, but this reference strain could produce strong yellowish pink and dark yellowish pink diffusible pigments on ISP 5 and tryptic soy agar, respectively; NaCl tolerance of these two strains was up to 5.0 %, which is lower than that of S. violaceorectus DSM 40279T (6.0 %); and both strains could grow at pH 11.0 while S. violaceorectus could...
Other phenotypic differences included production of H₂S, hydrolysis of aesculin and utilization of L-arabinose, D-fructose, D-mannitol, L-rhamnose, dulcitol, L-asparagine and L-arginine. Moreover, some chemotaxonomic characteristics, such as the absence of hydroxy-phosphatidylethanolamine, phosphatidylinositol and MK-9(H₄), could also distinguish the two isolates from *S. violaceorectus* DSM 40279ᵀ (Table 1, Fig. S1). Therefore, it is evident from the phenotypic, genotypic and chemotaxonomic data that strains NEAU-A4ᵀ and NEAU-A3 represents one novel species of the genus *Streptomyces*, for which the name *Streptomyces tritici* is proposed.

**DESCRIPTION OF STREPTOMYCES TRITICI SP. NOV.**

*Streptomyces tritici* (tri'ti.ci. L. gen. n. *tritici* of *Triticum*, referring to the isolation of the organism from rhizosphere soil of *Triticum aestivum* L.).
Table 1. Differential characteristics of strains NEAU-A4<sup>1</sup>, NEAU-A3 and S. violaceorectus DSM 40279<sup>1</sup>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Hydrolysis of aesculin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Production of H&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Liquefaction of gelatin</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>6–11</td>
<td>6–11</td>
<td>6–10</td>
</tr>
<tr>
<td>NaCl tolerance range (w/v, %)</td>
<td>0–5</td>
<td>0–5</td>
<td>0–6</td>
</tr>
<tr>
<td>Carbon source utilization</td>
<td>L-Arginine</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>D-Galactose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>L-D-Mannitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>L-Rhamnose</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>L-Xylose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrogen source utilization</td>
<td>L-Arginine</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>L-Aspartic acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>L-Asparagine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>L-Tyrosine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Menaquinones</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>MK-10(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>DPG, PE, PIM, L</td>
<td>DPG, PE, PIM, L</td>
<td>DPG, PE, OH, PE, PI, PIM, PLs</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10 %)</td>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt;-anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt;-anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>iso-C&lt;sub&gt;16:0&lt;/sub&gt;-iso-C&lt;sub&gt;16:0&lt;/sub&gt;, anteiso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; OH-PE, hydroxy-phosphatidylethanolamine; PIM, phosphatidylinositol mannoside; PL, unidentified phospholipid; L, unidentified lipid.

Gram-stain-positive, aerobic actinomycete that forms well-developed, branched substrate hyphae and aerial mycelium that differentiates into straight or flexuous spore chains consisting of cylindrical spores (0.6–0.9×1.0–1.9 µm), the spore surface is wrinkled or rough. Good growth on ISP 1, ISP 2, ISP 3, ISP 4, ISP 6, Bennett’s agar, Czapek’s agar, nutrient agar and tryptic soy agar; moderate growth on ISP 5 medium, and poor growth on ISP 7 medium. Greenish black diffusible pigment is observed on ISP 4 medium. Growth occurs at pH values between 6.0 and 11.0, the optimum being pH 7.0. Tolerates up to 5.0 % NaCl and grows optimally in 0–1 % (v/v) NaCl. Growth is observed at temperatures between 18 and 40 °C, with an optimum temperature of 28 °C. Positive for hydrolysis of starch and coagulation and peptonization of milk, but negative for reduction of nitrate, decomposition of Tweens (20, 40 and 80) and cellulose and production of urease. Strains 1–3 could utilize D-glucose, inositol, raffinose, D-sorbitol and sucrose as sole carbon sources but not D-mannose or D-ribose. All strains could utilize L-alanine, creatine, L-glutamic acid, glycine, L-glutamine, L-proline, L-serine and L-threonine as sole nitrogen sources. All strains contained ribose and glucose as whole-cell sugars. All data are from this study and the media used for conducting comparison tests for S. violaceorectus DSM 40279<sup>1</sup> are the same as those for NEAU-A4<sup>1</sup> and NEAU-A3.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**References**
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