**Glycomyces rhizosphaerae** sp. nov., isolated from the root and rhizosphere soil of wheat (*Triticum aestivum* L.)

Wenchao Li,1 Junwei Zhao,1 Linlin Shi,1 Jiabin Wang,1 Han Wang,1 Xiangjing Wang1,2,* and Wensheng Xiang1,2,*

**Abstract**

Two actinomycete strains, NEAU-C11T and NEAU-C8, isolated from rhizosphere soil and wheat root, respectively, collected from Langfang, Hebei Province, China. A polyphasic study was carried out to establish the taxonomic position of the two strains. Morphological and chemotaxonomic characteristics of the isolates coincided with the genus *Glycomyces*. Sequences analysis of the 16S rRNA gene also showed that the organisms belong to the genus *Glycomyces* and *Glycomyces algeriensis* is the highest sequence match for both strains. Furthermore, a combination of DNA–DNA hybridization results and some different physiological and biochemical properties indicated that they were distinguishable from the phylogenetically closest relatives. Therefore, the two strains represent a novel species, for which the name *Glycomyces rhizosphaerae* sp. nov. is proposed. The type strain is NEAU-C11T (=CGMCC 4.7396T=DSM 104646T).

The genus *Glycomyces*, which belongs to the family *Glycomycetaceae* of the order *Glycomycetales*, was originally established by Labeda *et al.* [1] and the description was later emended by Labeda and Kroppenstedt [2]. At the time of writing, 17 species with validly published names (www.bacterio.net/-allnamesdl.html) have been described. During the investigation of novel actinomycetes from wheat root and rhizosphere soil in Hebei Province, China, we isolated two strains, NEAU-C11T and NEAU-C8, which showed typical morphological characteristics of the genus *Glycomyces* but clearly different from related species of the genus *Glycomyces*. Here we report on the taxonomic characterization and propose a novel species, *Glycomyces rhizosphaerae* sp. nov., for the two strains.

Strains NEAU-C11T and NEAU-C8 were isolated from rhizosphere soil and wheat root, respectively. Strains were isolated from root as described by Liu *et al.* [3] and from soil as described by Li *et al.* [4]. The novel strains were routinely cultivated on International Streptomycetes Project (ISP) 3 agar [5] at 28 °C. Morphological observations were made using the media ISP 2–7, nutrient agar (NA), modified Bennett’s agar (MBA) and Czapek’s agar (CA) [5–8]. Colour determination was done with colour chips from the International Color Council–National Bureau of Standards colour charts [9]. Scanning electron microscopy was performed using cultures grown on ISP 3 agar at 28 °C for 4 weeks. Spore motility was assessed by light microscopic (Nikon Eclipse E200) observation of cells suspended in phosphate buffer (pH 7.0, 1 mM). 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 and 7 %, w/v) were tested in glucose–yeast extract powder (GY) medium [10] 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 [11]. The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, coagulation and peptonization of milk, and liquefaction of gelatin were examined as described previously [12, 13].

Biomass for chemical studies was prepared by growing the organism in GY medium in shake flasks at 28 °C for 7 days. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. Two novel strains were investigated to determine the type of diamino acid in cell-wall hydrolysates and analysed by a high-performance liquid chromatography (HPLC) method [14]. The whole-cell sugars were...
detected according to the procedures developed by Lechevalier and Lechevalier [15]. Polar lipids were extracted and examined by two-dimensional thin-layer chromatography and identified using the procedures of Minnikin et al. [16]. Menaquinones were extracted using the method of Collins et al. [17] and analysed by HPLC [18]. Cellular fatty acid composition was determined as described by Gao et al. [19] and analysed by gas chromatography–mass spectroscopy using the method of Xiang et al. [20]. Mycolic acids were checked by following the acid methanolysis method of Minnikin et al. [21].

Genomic DNA extraction and PCR amplification of the 16S rRNA gene from strains NEAU-C11T and NEAU-C8 were performed according to an established method [22]. Multiple alignments with sequences from the most closely related species of the genus Glycomyces and calculations of sequence similarity were carried out on the EzBioCloud server [23]. Phylogenetic analysis was performed with two tree-making algorithms (neighbour-joining [24] and maximum-likelihood [25]) using the software package MEGA version 6.06 [26]. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein [27] with 1000 replicates. 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). The G+C contents of the genomic DNA were determined by using the thermal denaturation (Tm) method [29] with Escherichia coli JM109 DNA used as a control. The DNA–DNA relatedness test between strains was carried out as described by De Ley et al. [30] under consideration of the modifications described by Huss et al. [31], using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thristomatted 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× saline sodium citrate (SSC) 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 replications and the DNA–DNA relatedness value was expressed as mean of the three values.

The physiological and biochemical differences between strains NEAU-C11T/NEAU-C8 and related type strains are presented in Table 1. Both strains produced well-developed and branched substrate hyphae on ISP 2 medium, but no aerial hyphae. Spore chains were straight and spores were cylindrical and non-motile (0.35–0.42×0.6–0.8 μm) (Fig. S1, available in the online version of this article). The colour of the substrate mycelium was yellowish white to dull orange on ISP 2, ISP 5, ISP 6, ISP 7, CA, MBA and NA media, and yellowish grey on ISP 3 and ISP 4 media. No diffusible pigment was observed on any of the tested media.

<table>
<thead>
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<th>Test</th>
<th>NEAU-C11T</th>
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<td>L: Rhamnose</td>
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<td>D: Raffinose</td>
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<td>D: Galactose</td>
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<td>L: Asparagine</td>
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</table>

Strains NEAU-C11T and NEAU-C8 had identical chemotaxonomic characteristics, and they were similar to many members of the genus *Glycomyces*. Both strains were found to contain meso-diaminopimelic acid as a diamino acid. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol and phosphohepglipolipid (Fig. S2). The whole-cell hydrolysates were determined to contain galactose, xylose and glucose. The menaquinones of strains NEAU-C11T and NEAU-C8 were identified as MK-10(H4) (53.6 %), 98.2 % to *Glycomyces harbinensis* IFO 14487T (All data were determined in this study. +, Positive; w, weak; –, negative.

A BLASTn analysis of their 16S rRNA gene sequences showed that the strains belonged to the genus *Glycomyces*. EzBioCloud server showed that strain NEAU-C11T was 98.6 % similar to *Glycomyces algeriensis* NRRL B-16327T, 98.4 % to *Glycomyces lechevalierae* NRRL B-16149T, 98.3 % to *Glycomyces endophyticus* YIM 56134T, 98.2 % to *Glycomyces rutgersensis* IFO 14488T and 98.1 % to *Glycomyces harbinensis*
IFO 14487\textsuperscript{T}, whilst strain NEAU-C8 was 98.8, 98.5, 98.4, 98.3 % similar to these type strains, respectively. Organisms NEAU-C11\textsuperscript{T} and NEAU-C8 shared 99.41 % 16S rRNA gene sequence similarity, and showed the highest similarities to \textit{G. algeriensis}. The phylogenetic tree based on the neighbour-joining algorithm (Fig. 1) showed that strains NEAU-C11\textsuperscript{T} and NEAU-C8 formed a monophyletic line within the genus \textit{Glycomyces}, and clustered with \textit{G. algeriensis} NRRL B-16327\textsuperscript{T}, \textit{G. lechevalierae} NRRL B-16149\textsuperscript{T}, \textit{G. endophyticus} YIM 56134\textsuperscript{T}, \textit{G. rutgersensis} IFO 14488\textsuperscript{T} and \textit{G. harbinensis} IFO 14487\textsuperscript{T}, which was supported by a bootstrap value of 99 % and also recovered by the maximum-likelihood algorithm (Fig. S3). DNA–DNA hybridization was used to further clarify the relatedness between the two strains and their phylogenetic relatives. The results showed that the DNA relatedness of the two isolates was 79.3±4.0 %; the values of DNA relatedness between strains NEAU-C11\textsuperscript{T} and NEAU-C8 were 72.0±0.2 and 71.8±0.2 mol\%, respectively. Although both isolates had the highest 16S rRNA gene sequence similarity to \textit{G. algeriensis}\textsuperscript{100}, the DNA hybridization values showed that they were more closely related to \textit{G. endophyticus} isolated from the root of a medicinal plant.

Examining strain NEAU-C11\textsuperscript{T} further as a type strain and representative example of the both strains showed typical characteristics consistent with NEAU-C8. Compared with the closely related species of the genus \textit{Glycomyces}, the selected strain, NEAU-C11\textsuperscript{T}, could be distinguished by physiological and biochemical characteristics as summarized in Table 1, such as maximum temperature for growth, nitrate reduction and utilization of L-asparagine, D-fructose, D-galactose, D-rafinose, L-rhamnose and L-serine. Moreover, strains NEAU-C11\textsuperscript{T} and NEAU-C8 could be differentiated from other type species by cultural characteristics on ISP 3 and ISP 4 (Fig. S4).

Based on the phenotypic and genotypic data, strains NEAU-C11\textsuperscript{T} and NEAU-C8 can be distinguished from their closest phylogenetic relatives, and proposed to represent a new species in the genus \textit{Glycomyces}, for which the name \textit{Glycomyces rhizosphaerae} sp. nov. is proposed.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{phylogenetic_tree.png}
\caption{Neighbour-joining tree showing the phylogenetic position of strains NEAU-C11\textsuperscript{T} and NEAU-C8 and their nearest neighbours based on 16S rRNA gene sequences. Only bootstrap values above 50 % (percentages of 1000 replications) are indicated. Asterisks indicate branches also recovered in the maximum-likelihood tree. Bar, 0.02 nucleotide substitutions per site.}
\end{figure}
**DESCRIPTION OF GLYCOMYCES RHIZOSPHAERAE SP. NOV.**

Glycomyces rhizosphaerae (rhiz.o.spha.‘e.ρae. N.L. gen. n. rhizo.sphaerae, of the rhizosphere).

Aerobic, Gram-stain-positive actinomycete which forms abundant substrate mycelia but no aerial mycelia. Good growth occurs on ISP 2, ISP 3, ISP 4, ISP 5, ISP 7 and CA media, poor growth on ISP 6, MBA and NA media. No diffusible pigment is observed on any of the media tested. Growth occurs at pH values between 7.0 and 11.0, the optimum pH 7.0. Tolerates up to 5.0 % NaCl and grows at temperatures between 15 and 40°C, with an optimum temperature of 28°C. Positive for hydrolysis of starch, liquefaction of gelatin, decomposition of Tweenes (40 and 80) and negative for coagulation and peptonization of milk, production of H$_2$S, urease and cellulase and reduction of nitrate. Hydrolysis of aesculin and decomposition of cellulose are positive or negative. L-Arabinoce, D-Fructose, D-glucose, inositol, lactose, maltose, D-mannitol, D-raffinose, L-rhamnose, D-sorbitol and sucrose are utilized as sole carbon sources, but not D-ribose or D-xylene. L-Aspartic acid, L-glutamic acid, L-proline, L-serine, and L-threonine are utilized as sole nitrogen sources, but not L-alanine, glycine or L-tyrosine. D-Galactose, creatine and L-asparagine are utilized or not. Cell walls contain meso-diaminopimelic acid and the whole-cell hydrolysates are galactose, xylose and glucose. The polar lipids contain diphosphatidylglycerol, phosphatidylinositol, lactose, maltose, -asparagine are utilized or not. Cell walls contain D-glutamic acid, glycine or L-tyrosine. D-L-Aspartic acid, L-glutamic acid, L-proline, L-serine, and L-threonine are utilized as sole nitrogen sources, but not L-alanine, glycine or L-tyrosine. D-Galactose, creatine and L-asparagine are utilized or not. The predominant menaquinones are MK-10(H$_6$) and MK-10(H$_2$). Major fatty acids (>10 %) are anteiso-C$_{15:0}$ iso-C$_{16:0}$, anteiso-C$_{17:0}$ and iso-C$_{15:0}$.

The type strain is NEAU-C11T (=CGMCC 4.7396T=DSM 104646T), isolated from rhizosphere soil of wheat (Triticum aestivum L.) collected from Langfang, Hebei Province, Central China. The DNA G+C content of the type strain is 71.8–72.0 mol%.

**Funding information**

This work was supported in part by grants from the National Natural Science Foundation of China (No. 31471832), Chang Jiang Scholar Candidates Program for Provincial Universities in Heilongjiang (CSCP).

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


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