Glycomyces artemisiae sp. nov., an endophytic actinomycete isolated from the roots of Artemisia argyi

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An endophytic actinomycete strain, IXS4T, was isolated from the root of Artemisia argyi, a medicinal plant collected from Yesanpo located in Laishui county, Hebei province, China. The 16S rRNA gene sequence of strain IXS4T showed most similarity to Glycomyces mayteni YIM 61331T (98.23 % 16S rRNA gene sequence similarity), Glycomyces scopariae YIM 56256T (98.00 %), Glycomyces sambucus E71T (97.90 %) and Glycomyces algeriensis NRRL B-18327T (97.10 %). DNA–DNA hybridization values between strain IXS4T and the closely related type strains were well below 70 %. The strain also showed a number of physiological and biochemical characteristics that were distinct from the closely related species. The strain contained MK-10(H2) and MK-11(H0) as the detected menaquinones. The peptidoglycan was mainly meso-diaminopimelic acid and the whole-cell sugars contained galactose, glucose, mannose, xylose and ribose. The major cellular fatty acids were iso-C14:0, iso-C15:0, iso-C16:0, anteiso-C15:0 and anteiso-C17:0. Based on the genetic and phenotypic properties, it is proposed that strain IXS4T represents a novel species of the genus Glycomyces, with the name http://dx.doi.org/10.1601/nm.7671 Glycomyces artemisiae sp. nov. The type strain is IXS4T (=HBUM178000T=CGMCC 4.7067T=NRBC 109773T).

The genus Glycomyces was initially established by Labeda et al. (1985), and at the time of writing, it holds 11 species with validly published names. While most strains have been isolated from soil collected in diverse geographical regions (Labeda & Kroppenstedt, 2004; Guan et al., 2011; Labeda et al., 1985; Evtushenko et al., 1991), members of the genus Glycomyces also appear to be closely associated with plant tissues (Gu et al., 2007; Qin et al., 2008, 2009). In the present paper, we describe the phenotypic and genotypic characterization of a Glycomyces-like strain (IXS4T), isolated from the roots of Artemisia argyi, a medicinal plant collected from Yesanpo in Hebei province, north China (115° 27’ E 39° 40’ N) at an elevation of 1980 m.

Healthy root samples of Artemisia argyi were used as sources for isolation of endophytic actinomycetes. The fresh root samples were air-dried for 48 h and then washed thoroughly as described by Coombs & Franco (2003). Subsequently, the root samples were excised and subjected to a modified five-step surface-sterilization procedure according to Qin et al. (2008): a 3 min wash in 5 % NaOCl, followed by a 10 min wash in 2.5 % Na2S2O3, a 3 min wash in 75 % ethanol, a 10 min wash in 10 % NaHCO3 and a final rinse in sterile distilled water. Surface-sterilized roots were pulverized in a ceramic mortar, distributed on tap water-yeast extract medium (Crawford et al., 1993) and incubated at 28 °C for 3 weeks. Isolates were purified and maintained on yeast extract-malt extract agar [International Streptomyces Project (ISP) medium 2] (Shirling & Gottlieb, 1966) at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene from strain IXS4T was performed as described by Coombs & Franco (2003). The PCR products were purified and cloned into the pMD 18-T vector (TaKaRa) and sequenced by using an Applied Biosystems DNA sequencer (model 3730XL) and the software provided by the manufacturer. The nearly full-length 16S rRNA gene sequence (1487 bp) was aligned manually in the GenBank database with BLAST (Altschul et al., 1990), which showed that strain IXS4T was closely related to members of the genus Glycomyces. MEGA software version 5.0 (Tamura et al., 2011) was used for the multiple alignment and for the phylogenetic tree reconstruction using the neighbour-joining (Saitou &
Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Felsenstein, 1981; Fitch, 1971) tree-making algorithms. Evolutionary distance matrices were calculated according to the neighbour-joining method with Kimura’s two-parameter model (Kimura, 1980). The robustness of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. 16S rRNA gene sequence similarities between the strains were calculated on the basis of pairwise alignment using the EzTaxon server ([http://ijs.sgmjournals.org](http://ijs.sgmjournals.org); Kim et al., 2012). Strain IXS4T was closely related to *Glycomyces mayteni* YIM 61331T (98.23 % 16S rRNA gene sequence similarity), *Glycomyces scopariae* YIM 56256T (98.00 %), *Glycomyces sambucus* E71T (97.90 %) and *Glycomyces algeriensis* NRRL B-16327T (97.10 %); the similarities between strain IXS4T and other members of the *Glycomyces* were all below 97 %. In the phylogenetic trees, the affiliation between strain IXS4T and its closest neighbour, *G. mayteni* YIM 61331T, was supported with bootstrap values of 53 %, 67 % and 70 % in the neighbour-joining (Fig. 1), maximum-likelihood (Fig. S1, available in the online Supplementary Material) and maximum-parsimony (Fig. S2) trees, respectively, and the node branches were identical in two different algorithms.

The liquid reassociation rate method (De Ley et al., 1970) was used for determining the percentage of DNA–DNA hybridization, in duplicate, by using a spectrophotometer (model CE9500; Cecil Instruments) equipped with a programmable melting-temperature control unit. The DNA–DNA relatedness values between strain IXS4T and *G. mayteni* YIM 61331T, *G. scopariae* YIM 56256T, *G. sambucus* E71T and *G. algeriensis* NRRL B-16327T were 59.4 %, 24.3 %, 20.2 % and 8.6 %, respectively, all of which are below the 70 % cut-off point for recognition of genomic species (Wayne et al., 1987; Stackebrandt & Goebel, 1994).

The DNA G+C content of strain IXS4T was determined by HPLC (Mesbah et al., 1989) to be 74 mol%, which is marginally higher than other members of the genus (70–72 mol%) (Guan et al., 2011; Gu et al., 2007; Qin et al., 2008; Qin et al., 2009).

Biomass for chemotaxonomic studies were obtained by growth in ISP 2 broth or TSB (trypticase soy broth) for fatty acids, at 28 °C for 14 days on a rotary shaker till stationary phase, and harvested by centrifugation and washed with distilled water. Diaminopimelic acid (DAP) was analysed by TLC (Bousfield et al., 1985) and whole-cell sugars were analysed by TLC (Hasegawa et al., 1983). Meso-DAP and a minor amount of L-DAP were present, and the whole-cell sugars contained galactose, glucose, mannose, xylose and a minor amount of ribose (Fig. S3), while the whole-cell sugars of the closest related type strain, *G. mayteni* YIM 61331T, did not contain mannose and only contained meso-DAP. The isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by using TLC on Gel 60 F254 plates (10×20 cm, 0.5 mm thickness) and using n-hexane/diethyl ether (85:15, v/v) as the solvent. The identities of the quinones were analysed by HPLC with a Cosmosil SC18column (4.6×250 mm; Thermo Electron Corporation). Strain IXS4T contained MK-10(H3) as the predominant menaquinone, with MK-11(H6) also present (Fig. S4). The menaquinone profile of the closest related type strain, *G. mayteni* YIM 61331T, was very different, it contained MK-11(H6), MK-11(H4) and MK-10(H4) (Qin et al., 2009). Detailed menaquinone data for the novel strain and related species of the genus *Glycomyces* are given in Table S1.

Polar lipids were extracted and identified by two-dimensional TLC (silica gel 60, 10×10 cm) as described by Minnikin et al. (1984). Major lipids were diphasphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, diphasphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, diphasphatidylglycerol, phosphatidylglycerol, and phosphatidylcholine.
phosphatidylinositol, phosphatidylinositol mannosides and an unidentified phospholipid (Fig. S5).

For the analysis of whole-cell fatty acids, strain IXS4 was grown for 14 days at 28 °C in TSB (trypticase soy broth) in an Erlenmeyer flask at 180 r.p.m., and harvested by centrifugation. The washed cells (100 mg) were saponified, methylated and extracted, and the fatty acid methyl esters (FAMEs) were determined using the standard MIDI (Microbial Identification System, Sherlock version 6.1) procedure (Sasser, 2009) and a gas chromatograph (7890A GC system; Agilent). The resulting profiles were identified using the TSBA6 database, version 6.1. The whole-cell fatty acid pattern of strain IXS4 was of the iso-anteiso-branched type (Table S2). The major cellular fatty acid of strain IXS4 was anteiso-C15:0 which is different from the one of iso-C16:0 in the closest related type strain, G. mayteni YIM 61331T. In addition, the other major fatty acids detected in strain IXS4 were iso-C16:0 anteiso-C17:0, iso-C15:0 and iso-C14:0. Detailed differences in fatty acids between the novel strain and related species of the genus Glycomyces are given in Table S2.

Cultural characteristics were observed on the media of Shirling & Gottlieb (1966), potato-dextrose agar (PDA; Difco), Czapek’s agar (Waksman, 1967) and nutrient agar (Waksman, 1967). Colours and hues were determined to those of G. mayteni YIM 61331T. The strain did not show cultural and morphological characteristics similar to those of G. mayteni YIM 61331T. The strain did not produce diffusible pigments on any of the media used. On most media, it formed oyster white to yellowish-white substrate mycelium and abundant white aerial mycelia that sprouted into spherical bulges then became rod-like bulges (Fig. S6) and formed branching hyphae in the end. Waxy transparent capsule outside the hyphae (Fig. S6) and formed branching hyphae in the end. After 9 days growth, aerial mycelia fractured and formed a transparent capsule outside the hyphae (Fig. S6).

Acid production from carbohydrates, decomposition of test substances and utilization of sole carbon sources for energy and growth were examined by the methods of Gordon et al. (1974). Growth at different temperatures (4, 15, 20, 28, 37, 40 and 42 °C), NaCl concentrations (0–10 % at intervals of 0.5 % NaCl, w/v) and pH (pH 4.0–11.0 at intervals of 1 pH unit) was assessed after incubation at 28 °C for 7–14 days on ISP 2 medium. The physiological properties of strain IXS4 and its closest neighbour, G. mayteni YIM 61331T, were significantly different in terms of acid production from D-mannitol, D-galactose, sucrose and maltose, assimilation of acetate, succinate, propionate and tartrate, decomposition of xanthine and L-tyrosine, and reduction of nitrate (Table 1). Detailed physiological characteristics of strain IXS4 are given in Table 1 and the species description.

Based on the results of this study, isolate IXS4 is proposed to represent a novel species of the genus Glycomyces, with the name Glycomyces artemisiae sp. nov.

Table 1. Differential characteristics of strain IXS4 and type strains of related species of the genus Glycomyces

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<td>Acid production from:</td>
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**Description of Glycomyces artemisiae sp. nov.**

Glycomyces artemisiae (ar.te.mi’si.ae. N. L. n. Artemisia a botanical genus name; N.L. gen. n. artemisiae of Artemisia, referring to the isolation of the type strain from tissues of Artemisia argyi).

Aerobic actinomycete that forms yellowish-white (on ISP 2 and Czapek’s agar) to orange-yellow (on ISP 3, ISP 4, ISP5, PDA and nutrient agar) substrate mycelium and white aerial mycelium that sprouted into spherical and rod-like bulges, then formed branching hyphae in the end. Waxy and plicate growth on most media. No soluble pigments are produced. The temperature range for growth is 20–40 °C, with optimal growth at 28 °C. The NaCl concentration range for growth is 0–5 % and the pH range for growth is pH 5.0–9.0. Additional physiological properties are given in Table 1. The cell wall contains meso-DAP. The whole-cell sugar pattern consists of galactose, glucose, mannose, xylose and ribose. The predominant menaquinones are

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MK-10(H2) and MK-11(H0). The major cellular fatty acids of strain IXS4T are anteiso-C15:0, anteiso-C17:0, iso-C16:0, iso-C15:0 and iso-C14:0.

The type strain, IXS4T (\(^{=}\)HBUM178000\(^{=}\)=CGMCC 4,7067\(^{=}\)=NBRC 109773\(^{=}\)), was isolated from surface-sterilized roots of Artemisia argyi collected from Yesanpo located in Laishui county, Hebei province, north China. The G+C content of genomic DNA of the type strain is 74 mol%.

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References


