Glycomyces scopariae sp. nov. and *Glycomyces mayteni* sp. nov., isolated from medicinal plants in China

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Two actinomycete strains, designated YIM 56256^T and YIM 61331^T, were isolated from the roots of *Scoparia dulcis* and *Maytenus austroyunnanensis*, two Chinese medicinal plants, and their taxonomic status was established based on a polyphasic investigation. The organisms were found to have chemical and morphological markers typical of members of the genus *Glycomyces*. 16S rRNA gene sequence analysis showed that they were closely related to each other and to *Glycomyces sambucus* E71^T. A battery of physiological characteristics and levels of DNA–DNA relatedness indicated that strains YIM 56256^T and YIM 61331^T represent two novel species, clearly different from the related known *Glycomyces* species. On the basis of the data presented, it is evident that each of these strains represents a novel species of the genus *Glycomyces*, for which the names *Glycomyces scopariae* sp. nov. (type strain YIM 56256^T = KCTC 19158^T = DSM 44968^T) and *Glycomyces mayteni* sp. nov. (type strain YIM 61331^T = KCTC 19527^T = CCTCC AA 208004^T) are proposed.

The genus *Glycomyces* was established by Labeda *et al.* (1985) and the description of the genus was emended by Labeda & Kroppenstedt (2004) on the basis of chemotaxonomic data. At the time of writing, the genus *Glycomyces* comprises eight species with validly published names, *Glycomyces algeriensis*, *G. arizonensis*, *G. harbinensis*, *G. lechevalierae*, *G. rutgersensis*, *G. tenuis*, *G. sambucus* and *G. endophyticus* (Labeda *et al.*, 1985; Evtushenko *et al.*, 1991; Labeda & Kroppenstedt, 2004; Gu *et al.*, 2007; Qin *et al.*, 2008). The last of these species was proposed for a strain isolated during the course of a study on endophytic actinomycete diversity from tropical rainforest medicinal plants (Qin *et al.*, 2008); here, we report on another two novel *Glycomyces*-like strains from this study, designated YIM 56256^T and YIM 61331^T. The aim of this study was to

determine the exact taxonomic position of the two strains by means of a polyphasic characterization.

Healthy root samples of *Scoparia dulcis* and *Maytenus austroyunnanensis*, two traditional Chinese medicinal plants, were collected from a tropical rainforest in Xishuangbanna, Yunnan province, south-west China, and used as sources for isolation of endophytic actinomycetes. The plant samples were air-dried at room temperature after thorough washing under tap water and then surface-sterilized according to the five-step sterilization procedure (Qin *et al.*, 2008). 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 (ISP medium 2; Shirling & Gottlieb, 1966) at 4 °C and as glycerol suspensions (20 %, v/v) at –20 °C.

Cultural characteristics were observed on potato-dextrose agar (PDA; Difco), Czapek's agar (Waksman, 1967), nutrient agar (Waksman, 1967) and International *Streptomyces* Project media ISP 2, ISP 3, ISP 4 and ISP 5 after 7, 14, 21 and 28 days at 28 °C according to the methods of Shirling & Gottlieb (1966). The colours of both

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM 56256^T and YIM 61331^T are EU200682 and EU814511.

Scanning electron micrographs of strains YIM 56256^T and YIM 61331^T and comparisons of fatty acid and menaquinone profiles are available as supplementary material with the online version of this paper.

substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the colour charts of the Inter-Society Color Council–National Bureau of Standards (Kelly, 1964). Morphological characteristics were observed by using light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after 21 days growth on ISP 2 medium. The temperature range (0, 4, 10, 15, 20, 28, 37, 40, 45 and 55 °C), pH range (pH 4.0–11.0 at intervals of 0.5 pH units) and requirement for NaCl (0–15 %, w/v, at intervals of 0.5 %) were determined by using ISP 2 as the basal medium. Media and procedures used for determination of physiological features including acid production from carbohydrates, decomposition of test substances and utilization of sole carbon sources for energy and growth were those described by Gordon *et al.* (1974). Detailed physiological characteristics of strains YIM 56256^T and YIM 61331^T are given in Table 1 and the species descriptions.

Biomass for most of the chemotaxonomic studies was obtained after cultivation at 28 °C for 7–10 days in shaken cultures with ISP 2 broth. Amino acid and sugar analysis of whole-cell hydrolysates was performed according to the procedures described by Hasegawa *et al.* (1983). Polar lipids were extracted, examined using two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Cellular menaquinones were extracted and purified as described by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). Biomass for quantitative fatty acid analysis was prepared by scraping colonies from TSA plates that had been incubated for 10 days at 28 °C. Fatty acids were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Extraction of genomic DNA and amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). Almost-complete 16S rRNA gene sequences of strain YIM 56256^T and YIM 61331^T were obtained and BLAST search comparisons were made against the GenBank/EMBL/DDBJ databases. Subsequently, a phylogenetic analysis was performed using the software package MEGA 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). The phylogenetic tree was constructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods. The stability of relationships was assessed by performing bootstrap analyses (Felsenstein, 1985) of the neighbour-joining data based on 1000 resamplings. Determination of DNA G+C contents was performed according to Mesbah *et al.* (1989). Levels of DNA–DNA relatedness were determined according to the fluorometric micro-well method (Ezaki *et al.*, 1989; He *et al.*, 2005).

Strains YIM 56256^T and YIM 61331^T were aerobic and stained Gram-positive. They grew well on ISP 2 and ISP 4 media and Czapek's agar. On most media tested, they

Table 1. Differential characteristics of strains YIM 61331^T and YIM 56256^T and type strains of related *Glycomyces* species.

Strains: 1, YIM 61331^T; 2, YIM 56256^T; 3, *G. algeriensis* NRRL B-16327^T; 4, *G. harbinensis* IFO 14487^T; 5, *G. lechevalierae* DSM 44724^T; 6, *G. rutgersensis* IFO 14488^T; 7, *G. sambucus* DSM 45047^T; 8, *G. endophyticus* YIM 56134^T. +, Positive; –, negative; w, weakly positive. All strains are positive for acid production from L-arabinose, D-glucose, D-mannose, methyl α -D-glucoside, salicin and D-xylose, for decomposition of adenine and casein and for growth on 3 % NaCl. All strains are negative for acid production from dulcitol. Data for reference strains were taken from Labeda *et al.* (1985), Labeda & Kroppenstedt (2004), Gu *et al.* (2007) and Qin *et al.* (2008).

Characteristic	1	2	3	4	5	6	7	8
Acid produced from:								
D-Fructose	+	–	+	+	+	+	+	+
D-Rhamnose	–	–	+	+	+	+	+	+
D-Lactose	–	–	+	+	+	w	+	+
D-Mannitol	+	+	–	–	–	+	–	–
Melezitose	–	–	–	–	–	w	–	–
Raffinose	–	–	–	+	–	+	+	–
D-Sorbitol	–	–	–	+	w	+	–	–
D-Galactose	–	+	+	+	+	+	+	+
Cellobiose	–	+	+	+	+	+	+	+
Sucrose	+	–	–	–	+	–	+	+
Trehalose	+	–	–	+	+	+	–	w
Dextrin	+	–	+	+	+	+	+	+
Erythritol	–	+	–	+	–	–	–	–
Inositol	–	–	+	–	+	–	–	+
Maltose	+	+	w	+	+	+	+	+
Methyl β -xyloside	–	+	–	+	–	+	+	+
Melibiose	–	–	–	–	w	–	+	–
Adonitol	–	–	–	+	+	–	–	–
Assimilation of sole carbon sources								
Acetate	–	–	w	+	+	+	+	–
Citrate	–	–	–	+	+	–	w	–
Oxalate	–	–	w	–	+	–	–	–
Succinate	–	+	–	+	+	–	+	+
Malate	+	+	w	+	+	+	+	+
Propionate	–	+	w	+	–	+	–	w
Tartrate	–	–	–	–	–	–	+	–
Decomposition of:								
Hypoxanthine	–	+	–	+	+	+	–	+
Xanthine	+	–	–	–	–	–	–	–
Starch	+	–	–	+	+	+	+	+
Aesculin	+	+	–	+	+	+	+	+
Gelatin	–	–	–	–	–	+	–	–
Urea	+	+	–	–	–	–	–	+
L-Tyrosine	+	–	–	–	+	–	–	+
Reduction of nitrate	+	–	w	w	+	+	w	+
Growth at 42 °C	–	–	–	+	–	–	–	–
Growth on 5 % NaCl	+	+	+	+	+	+	–	+

formed abundant white aerial mycelium that fragmented into square-ended conidia and yellowish-white to orange–yellow or cream–yellow substrate mycelium. Differential

properties among YIM 56256^T, YIM 61331^T and recognized species of the genus *Glycomyces* are given in Table 1.

Strains YIM 56256^T and YIM 61331^T contained *meso*-diaminopimelic acid as the cell-wall diamino acid. Whole-cell hydrolysates contained galactose, glucose and xylose, which differed slightly from reports for recognized *Glycomyces* species (Labeda & Kroppenstedt, 2004) in that they contained glucose but not mannose. Polar lipid extracts contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and several unknown phosphoglycolipids. The major fatty acids detected (percentages of the total cellular fatty acids) from strains YIM 56256^T and YIM 61331^T were iso-C_{15:0} (19.2 and 8.6 %, respectively), anteiso-C_{15:0} (36.1 and 26.4 %), iso-C_{16:0} (21.3 and 37.8 %) and anteiso-C_{17:0} (11.3 and 19.2 %). The predominant menaquinones of strains YIM 56256^T and YIM 61331^T were MK-10(H₄) (60 %) and MK-11 (42 %), respectively, which clearly differentiated the two new isolates from known recognized *Glycomyces* species. Detailed fatty acid and menaquinone data for the two novel strains and related *Glycomyces* species are given in Supplementary Tables S1 and S2, available in IJSEM Online. The DNA G+C contents of strains YIM 56256^T and YIM 61331^T were 72.2 and 71.5 mol%, respectively.

Almost-complete 16S rRNA gene sequences were determined for strains YIM 56256^T (1387 bp) and YIM 61331^T (1415 bp). A preliminary comparison of the sequences against the GenBank database indicated that the two isolates belonged to the genus *Glycomyces*. In the neighbour-joining phylogenetic tree for *Glycomyces* species (Fig. 1), strains YIM 56256^T, YIM 61331^T and *G. sambucus* E71^T formed a separate branch with high bootstrap support. 16S rRNA gene sequence similarity between the two new isolates was 98.6 %. In addition, strains YIM 56256^T and YIM 61331^T showed highest sequence similarity to *G. sambucus* E71^T (99.7 and 98.7 %) and moderately low 16S rRNA gene sequence similarities of 97.1–97.4 % to *G. lechevalierae* NRRL B-16149^T, *G. rutgersensis* NBRC 14488^T, *G. harbinensis* NBRC 14487^T and *G. algeriensis* NRRL B-16327^T. To establish the precise taxonomic position of strains YIM 56256^T and YIM 61331^T, DNA–DNA hybridization studies were performed.

The DNA–DNA relatedness between strains YIM 56256^T and YIM 61331^T was 38 %, and levels between strain YIM 56256^T and *G. sambucus* CGMCC 4.3147^T, *G. lechevalierae* DSM 44724^T, *G. rutgersensis* DSM 43812^T, *G. harbinensis* DSM 46494^T and *G. algeriensis* DSM 44727^T were 58, 25, 21, 29 and 17 %, respectively, while the value between YIM 61331^T and *G. sambucus* CGMCC 4.3147^T was 42 %. All these values were significantly below 70 %, the cut-off point recommended by Stackebrandt & Goebel (1994) for the recognition of genomic species.

Thus, on the basis of the phenotypic and chemotaxonomic data, phylogenetic analysis and the DNA–DNA relatedness data presented above, it is proposed that strains YIM 56256^T and YIM 61331^T should be placed in the genus *Glycomyces* as the type strains of novel species, with the names *Glycomyces scopariae* sp. nov. and *Glycomyces mayteni* sp. nov., respectively.

Description of *Glycomyces scopariae* sp. nov.

Glycomyces scopariae (sco.pa'ri.ae. N.L. n. *Scoparia* a botanical genus name; N.L. gen. n. *scopariae* of *Scoparia*, referring to the isolation of the type strain from tissues of *Scoparia dulcis*).

Aerobic actinomycete that forms yellowish-white (on ISP 2, ISP 3 and ISP 4 media) to orange–yellow (on PDA and nutrient agar) substrate mycelium and white aerial mycelium that fragments into square-ended conidia. Waxy and plicate growth on most media. No soluble pigments are produced. Temperature range for growth is 20–37 °C, with optimal growth at 28 °C. The NaCl concentration range for growth is 0–5 % and the pH range for growth is pH 6.0–8.0. Additional physiological properties are given in Table 1. The cell wall contains *meso*-diaminopimelic acid. The whole-cell sugar pattern consists of galactose, glucose and xylose. The predominant menaquinones are MK-10(H₄), MK-11(H₄) and MK-11 (60 : 18 : 14 in the type strain). Major fatty acids (>5 %) are anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{15:0}, anteiso-C_{17:0} and iso-C_{14:0}. The G+C content of genomic DNA of the type strain is 72.2 mol%.

The type strain, YIM 56256^T (=KCTC 19158^T =DSM 44968^T), was isolated from surface-sterilized roots of

Fig. 1. Neighbour-joining tree showing the phylogenetic relationships among strains YIM 56256^T and YIM 61331^T and the type strains of related taxa based on 16S rRNA gene sequences. Numbers at branch nodes are bootstrap values (percentages of 1000 replicates). Asterisks indicate clades that were conserved when neighbour-joining and maximum-parsimony methods were used to construct phylogenetic trees. Bar, 0.01 substitutions per nucleotide position.

Scoparia dulcis collected from a tropical rainforest in Xishuangbanna, Yunnan province, south-west China.

Description of *Glycomyces mayteni* sp. nov.

Glycomyces mayteni (may'te.ni. N.L. n. *Maytenus* a botanical genus name; N.L. gen. n. *mayteni* of *Maytenus*, referring to the isolation of the type strain from tissues of *Maytenus austroyunnanensis*).

Aerobic actinomycete that forms yellowish-white (on ISP 2, ISP 3 and ISP 4 media) to cream–yellow (on ISP 5 and Czapek's agar) substrate mycelium and white aerial mycelium. Waxy and plicate growth on most media. Chains of square-ended conidia are produced on aerial mycelium (Supplementary Fig. S1). No soluble pigments are produced. Temperature range for growth is 15–37 °C, with optimal growth at 28 °C. The NaCl concentration range for growth is 0–5 % and the pH range for growth is pH 6.0–8.0. Additional physiological properties are given in Table 1. The cell wall contains *meso*-diaminopimelic acid. The whole-cell sugar pattern consists of galactose, glucose and xylose. The predominant menaquinones are MK-11, MK-11(H₄) and MK-10 (42:36:15 in the type strain). Major fatty acids (>5 %) are iso-C_{16:0}, anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{15:0}. The G+C content of genomic DNA of the type strain is 71.5 mol%.

The type strain, YIM 61331^T (=KCTC 19527^T =CCTCC AA 208004^T), was isolated from surface-sterilized roots of *Maytenus austroyunnanensis* collected from a tropical rainforest in Xishuangbanna, Yunnan province, south-west China.

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