Glycomyces sambucus sp. nov., an endophytic actinomycete isolated from the stem of *Sambucus adnata* Wall

Qiang Gu,^{1,2} Wen Zheng¹ and Ying Huang¹

Correspondence
Ying Huang
huangy@im.ac.cn

¹State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China

²Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China

An actinomycete, designated strain E71^T, was isolated from the stem of *Sambucus adnata* Wall, a Chinese medicinal plant, and subjected to a polyphasic taxonomic study. Phylogenetic analyses based on the 16S rRNA gene sequence showed that the organism was a member of the genus *Glycomyces*, and formed a distinct phyletic line distantly related to recognized species of the genus *Glycomyces*. Morphological and chemotaxonomic data supported the affiliation of strain E71^T to the genus *Glycomyces*. A number of physiological properties and a unique menaquinone profile allowed differentiation of the strain from related *Glycomyces* species. It is therefore proposed that strain E71^T represents a novel species of the genus *Glycomyces*, for which the name *Glycomyces sambucus* sp. nov. is proposed. The type strain is E71^T (=CGMCC 4.3147^T=DSM 45047^T).

The genus *Glycomyces* was initially established by Labeda *et al.* (1985) and the description was later emended by Labeda & Kroppenstedt (2004). It is the type genus of the family *Glycomycetaceae*, which also contains the genus *Stackebrandtia* (Labeda & Kroppenstedt, 2005), and belongs to the suborder *Glycomycineae* (Stackebrandt *et al.*, 1997). At the time of writing, the genus comprises six species, *Glycomyces algeriensis*, *Glycomyces arizonensis*, *Glycomyces harbinensis*, *Glycomyces lechevalierae*, *Glycomyces rutgersensis* and *Glycomyces tenuis* (Labeda *et al.*, 1985; Evtushenko *et al.*, 1991; Labeda & Kroppenstedt, 2004), which were all isolated from soil, and are characterized by a type II cell-wall composition (*meso*-diaminopimelic acid and glycine), whole-cell sugar pattern consisting of ribose, xylose, mannose and galactose, type PI phospholipids pattern with significant amounts of phosphatidylinositol mannosides, and predominant menaquinones containing 10, 11 and/or 12 isoprene units. During an investigation into the diversity of endophytic actinomycetes from Chinese medicinal plants, a new strain, E71^T, was isolated. The aim of the present study was to determine the taxonomic status of this organism.

Stem samples of *Sambucus adnata* Wall, a traditional Chinese medicinal plant, were collected in the rainforest of Jinghong Natural Reserve, Yunnan Province, China, and were surface-sterilized according to the method of Coombs & Franco (2003). Strain E71^T was isolated from the stem by using the procedure and medium described by Gu *et al.* (2006).

Genomic DNA extraction and PCR amplification of the 16S rRNA gene from strain E71^T were performed according to an established method (Chun & Goodfellow, 1995). The PCR product was purified and sequenced by using an Applied Biosystems DNA sequencer (model 3730XL) and software provided by the manufacturer. Preliminary comparison of the resultant sequence (1410 nt) against DDBJ/EMBL/GenBank databases by using a standard nucleotide–nucleotide BLAST search program (Altschul *et al.*, 1997) indicated that strain E71^T was closely related to members of the genus *Glycomyces* but only very distantly related to other taxa. The 16S rRNA gene sequence of strain E71^T was then aligned with corresponding sequences of the type strains of recognized species of the genus *Glycomyces* retrieved from GenBank by using MEGA software (Molecular Evolutionary Genetics Analysis) version 3.1 (Kumar *et al.*, 2004), and phylogenetic trees were constructed according to the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms in the same software. Evolutionary distances for the neighbour-joining algorithm were calculated with Kimura's two-parameter model (Kimura, 1980), and close-neighbour-interchange (search level=2, random additions=100)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain E71^T is DQ460469.

A scanning electron micrograph of cells of strain E71^T and tables detailing the fatty acid content and menaquinone profile of strain E71^T and related species of the genus *Glycomyces* are available with the online version of this paper.

was applied in the maximum-parsimony analysis. The topology of the tree was evaluated by bootstrap analysis (Felsenstein, 1985) on the basis of 1000 replications. It is evident from the resultant phylogenetic tree (Fig. 1) that strain E71^T forms a distinct monophyletic line within the genus *Glycomyces*, supported by the two treeing algorithms and by high bootstrap values. The strain is related most closely, albeit loosely, to *G. lechevalierae* NRRL B-16149^T, *G. algeriensis* NRRL B-16327^T, *G. harbinensis* IFO14487^T and *G. rutgersensis* IFO14488^T, with moderately low 16S rRNA gene sequence similarities of 97.2–97.1 %, and is related more distantly to the type strains of *G. arizonensis* and *G. tenuis*, with sequence similarities below 96 %.

Cultural characteristics were observed on the media of Shirling & Gottlieb (1966), modified Bennett's medium (Jones, 1949) and ATCC medium 172 (Cote *et al.*, 1984). Morphological characteristics were examined by scanning electron (FEI QUANTA) microscopy of 14-day cultures grown on ISP 2 and ATCC medium 172 agar. The new organism showed cultural and morphological characteristics similar to those of *G. rutgersensis*, except that no pigments were produced. On most media it formed yellowish-white to tan substrate mycelium and abundant white aerial mycelia that fragmented into square-ended conidia (see Supplementary Fig. S1 available in IJSEM Online).

Biomass for chemotaxonomic study was prepared by growing the strain in shake flasks of ISP 2 at 28 °C for 7 days, and harvested by centrifugation, washed with distilled water and freeze-dried. Standard methods were used for the extraction and analysis of the isomers of diaminopimelic acid (Hasegawa *et al.*, 1983), whole-cell sugars (Lechevalier & Lechevalier, 1980), *N*-acyl type of muramyl residue in the cell-wall peptidoglycan (Uchida *et al.*, 1999), menaquinones (Collins, 1985), polar lipids

(Minnikin *et al.*, 1984) and fatty acids (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). *G. lechevalierae* NRRL B-16149^T was used as a reference. Strain E71^T showed a range of chemotaxonomic properties in line with its inclusion within the genus *Glycomyces*. It contained meso-diaminopimelic acid as cell-wall diamino acid; diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and several phosphoglycolipids of unknown composition as major polar lipids; a glycolyl type of muramyl acid; and its fatty acid profile was composed mainly of 15-, 16- and 17-carbon iso and anteiso components. The whole-cell sugar pattern consisted of galactose, glucose, xylose and a minor amount of ribose, and was slightly different from that reported for recognized *Glycomyces* species (Labeda & Kroppenstedt, 2004) in that it contained glucose but not mannose. The predominant menaquinones, which were considered to be species-specific for *Glycomyces* (Labeda & Kroppenstedt, 2004), clearly differentiated strain E71^T from recognized *Glycomyces* species. Detailed fatty acid and menaquinone data for the new strain and related *Glycomyces* species are given in the formal species description below, and in Supplementary Tables S1 and S2 available in IJSEM Online. The G+C content of the genomic DNA of strain E71^T (70 mol%) was determined by using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* K12 as a control.

The physiological characteristics of strain E71^T, including acid production from carbohydrates, utilization of sole carbon sources for energy and growth, and decomposition of test substances, were assessed by using the media and methods of Gordon *et al.* (1974). Although the results demonstrated a few common physiological characteristics among strain E71^T and related species of the genus *Glycomyces*, the data enabled the new strain to be distinguished easily from the latter (Table 1). These data, together with the unique menaquinone profile, distinct position in the *Glycomyces* phylogenetic tree and relatively low 16S rRNA gene sequence similarities with the type strains of recognized *Glycomyces* species, support the designation of strain E71^T as a novel species of the genus *Glycomyces*, for which the name *Glycomyces sambucus* sp. nov. is proposed.

Description of *Glycomyces sambucus* sp. nov.

Glycomyces sambucus (sam'bu.cus. N.L. gen. n. *sambucus* of the plant genus *Sambucus*)

Aerobic actinomycete that forms yellowish-white to tan substrate mycelium, depending on the growth medium. White aerial mycelia are produced and fragment into square-ended conidia. No soluble pigments are produced. Acid is not produced from L-lactulose or L-sorbose. D-Cellobiose, L-fucose, D-lactose, maltose, D-mannose, D-rhamnose, D-sorbitol, trehalose, sucrose, glycerol, glycogen, L-arginine, L-leucine, L-ornithine, L-proline, L-tyrosine, L-valine, methyl α -D-glucoside and salicin are utilized as sole

Fig. 1. Phylogenetic tree of the genus *Glycomyces* based on 16S rRNA gene sequence analysis. The neighbour-joining and maximum-parsimony algorithms were both implemented in the software package MEGA version 3.1 (Kumar *et al.*, 2004). Bootstrap values based on 1000 replicates are listed as percentages; those inferred from the neighbour-joining algorithm are given above branches and those from the maximum-parsimony algorithm are below. Bar, 0.01 substitutions per nucleotide position.

Table 1. Physiological characteristics of strain E71^T and related type strains of *Glycomyces* species

Strains: 1, E71^T; 2, *G. algeriensis* NRRL B-16327^T; 3, *G. harbinensis* IFO 14487^T; 4, *G. lechevalierae* DSM 44724^T; 5, *G. rutgersensis* IFO 14488^T. +, Positive; –, negative; w, weakly positive. All strains are positive for acid production from L-arabinose, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, methyl α -D-glucoside, glycerol D-mannose, D-rhamnose, D-xylose and salicin, for decomposition of adenine and casein, and for growth on 3% NaCl. All strains are negative for acid production from dulcitol, for decomposition of urea or xanthine, and for assimilation of benzoate. Data for reference strains were taken from Labeda *et al.* (1985) and Labeda & Kroppenstedt (2004).

Characteristic	1	2	3	4	5
Acid produced from:					
D-Lactose	+	+	+	+	w
D-Mannitol	–	–	–	–	+
D-Melezitose	–	–	–	–	w
D-Raffinose	+	–	+	–	+
D-Sorbitol	–	–	+	w	+
Sucrose	+	–	–	+	–
Trehalose	–	–	+	+	+
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	+	–	+
Lactate	w	–	–	–	+
Mucate	+	w	–	+	–
Tartrate	+	–	–	–	–
Decomposition of:					
Hypoxanthine	–	–	+	+	+
L-Tyrosine	–	–	–	+	–
Starch	+	–	+	+	+
Aesculin	+	–	+	+	+
Gelatin	–	–	–	–	+
Reduction of nitrate	w	w	w	+	+
Growth on 5% NaCl	–	+	+	+	+
Growth at 42 °C	–	–	+	–	–

carbon source, but D-fructose, D-inulin, D-lactulose, D-mannitol, dulcitol, D-xylose, erythritol, inositol, L-alanine, L-methionine, L-phenylalanine, malonate, D-glutamic acid, D-sorbose and L-cysteine are not. D-Melezitose and D-raffinose are weakly utilized as sole carbon source. Temperature range for growth is 20–37 °C. Additional physiological properties are listed in Table 1. The whole-cell sugar pattern consists of galactose, glucose,

xylose and ribose. The predominant menaquinones are MK-11 (69%) and MK-11 (H₄) (26%). The fatty acid profile comprises major amounts of 16:0 iso (28.8%), 15:0 anteiso (26.2%) and 17:0 anteiso (18.0%), and minor amounts of 15:0 iso (8.2%), 14:0 iso (7.2%), 16:0 iso G (5.4%), 17:1 anteiso A (2.8%) and 17:0 iso (1.4%). The G+C content of the genomic DNA is 70 mol%. Other chemotaxonomic characteristics are typical of the genus *Glycomyces*.

The type strain, E71^T (=CGMCC 4.3147^T=DSM 45047^T), was isolated from the surface-sterilized stem of *Sambucus adnata* Wall collected in the rainforest of Jinghong Natural Reserve, Yunnan Province, China.

Acknowledgements

This work was supported by the Knowledge Innovation Project of the Chinese Academy of Sciences, and by a grant from Hisun Pharmaceutical Company. We are grateful to Professors D. P. Labeda (NRRL) and R. M. Kroppenstedt (DSMZ) for providing reference type strains, and to Professors Cheng-Lin Jiang and Li-Hua Xu (Yunnan University) for their help in plant sample collection.

References

- Altschul, S. F., Madden, T. L., Schffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Evol Microbiol* **45**, 240–245.
- Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Coombs, J. T. & Franco, C. M. M. (2003). Isolation and identification of actinobacteria from surface-sterilized wheat roots. *Appl Environ Microbiol* **69**, 5603–5608.
- Cote, R., Daggett, P.-M., Gant, M. J., Hay, R., Jong, S.-C. & Pienta, P. (1984). *ATCC Media Handbook*. Rockville, MD: American Type Culture Collection.
- Evtushenko, L. I., Tapytkova, S. D., Akimov, V. N., Semyonova, S. A. & Kalakoutskii, L. V. (1991). *Glycomyces tenuis* sp. nov. *Int J Syst Bacteriol* **41**, 154–157.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.
- Gu, Q., Luo, H., Zheng, W., Liu, Z. & Huang, Y. (2006). *Pseudonocardia oroxyli* sp. nov., a novel actinomycete isolated from the surface-sterilized *Oroxylum indicum* root. *Int J Syst Evol Microbiol* **56**, 2193–2197.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.

- Jones, K. L. (1949).** Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–145.
- Kämpfer, M. & Kroppenstedt, R. M. (1996).** Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kimura, M. (1980).** A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, S., Tamura, K. & Nei, M. (2004).** MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Labeda, D. P. & Kroppenstedt, R. M. (2004).** Emended description of the genus *Glycomyces* and description of *Glycomyces algeriensis* sp. nov., *Glycomyces arizonensis* sp. nov. and *Glycomyces lechevalierae* sp. nov. *Int J Syst Evol Microbiol* **54**, 2343–2346.
- Labeda, D. P. & Kroppenstedt, R. M. (2005).** *Stackebrandtia nassauensis* gen. nov., sp. nov. and emended description of the family *Glycomycetaceae*. *Int J Syst Evol Microbiol* **55**, 1687–1691.
- Labeda, D. P., Testa, R. T., Lechevalier, M. P. & Lechevalier, H. A. (1985).** *Glycomyces*, a new genus of the *Actinomycetales*. *Int J Syst Bacteriol* **35**, 417–421.
- Lechevalier, H. A. & Lechevalier, M. P. (1980).** The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy*, Special Publication no. 6, pp. 277–284. Edited by A. Dietz & D. W. Thayer. Arlington, VA: Society of Industrial Microbiology.
- Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984).** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997).** Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Uchida, K., Kudo, T., Suzuki, K. & Nakase, T. (1999).** A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49–56.