An actinomycete, designated strain E71T, 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 phylogenetic line distantly related to recognized species of the genus *Glycomyces*. Morphological and chemotaxonomic data supported the affiliation of strain E71T 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 E71T represents a novel species of the genus *Glycomyces*, for which the name *Glycomyces sambucus* sp. nov. is proposed. The type strain is E71T (=CGMCC 4.3147T=DSM 45047T).

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, E71T, was isolated. The aim of the present study was to determine the taxonomic status of this organism.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene from strain E71T 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 E71T was closely related to members of the genus *Glycomyces* but only very distantly related to other taxa. The 16S rRNA gene sequence of strain E71T 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 E71T is DQ460469.

A scanning electron micrograph of cells of strain E71T and tables detailing the fatty acid content and menaquinone profile of strain E71T and related species of the genus *Glycomyces* are available with the online version of this paper.

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

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

An actinomycete, designated strain E71T, 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 phylogenetic line distantly related to recognized species of the genus *Glycomyces*. Morphological and chemotaxonomic data supported the affiliation of strain E71T 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 E71T represents a novel species of the genus *Glycomyces*, for which the name *Glycomyces sambucus* sp. nov. is proposed. The type strain is E71T (=CGMCC 4.3147T=DSM 45047T).

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 E71T was isolated from the stem by using the procedure and medium described by Gu et al. (2006).
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 E71T forms a distinct monophyletic line within the genus Glycomyces, supported by the two tree-drawing algorithms and by high bootstrap values. The strain is related most closely, albeit loosely, to G. lechevalierae NRRL B-16149T, G. algeriensis NRRL B-16327T, G. harbinensis IFO14487T and G. rutgersensis IFO14488T, 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 dianomipelamic 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-16149T was used as a reference. Strain E71T 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, phosphatidylserine, 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 mannosate. The predominant menaquinones, which were considered to be species-specific for Glycomyces (Labeda & Kroppenstedt, 2004), clearly differentiated strain E71T 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 E71T (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 E71T, 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 E71T 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 E71T 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, glycophenol, L-arginine, L-leucine, L-ornithine, L-proline, L-tyrosine, L-valine, methyl a-D-glucoside and salicin are utilized as sole

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**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.
carbon source, but D-fructose, D-inulin, D-lactulose, 
D-mannitol, dulcitol, D-xylitol, 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, 
xylulose 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, E71T (=CGMCC 4.3147T=DSM 45047T), 
was isolated from the surface-sterilized stem of Sambucus 
adhata 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

Altenschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., 
generation of protein database search programs. Nucleic Acids Res 25, 
3389–3402.

Nocardia with 16S rRNA gene sequences. Int J Syst Evol Microbiol 45, 
240–245.

Collins, M. D. (1985). Isoprenoid quinone analysis in classification and 
Academic Press.

actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69, 
5603–5608.

Cote, R., Daggett, P.-M., Gantt, M. J., Hay, R., Jong, S.-C. & Pienta, P. 
Culture Collection.

Evteshenko, L. I., Tapytкова, S. D., Akimov, V. N., Semyonova, S. A. 

using the bootstrap. Evolution 39, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum 

(1974). Nocardia coeliaca, Nocardia autotrophica, and the nocardia 

oroxyli sp. nov., a novel actinomycete isolated from the surface-
sterilized Oxyryum indicum root. Int J Syst Evol Microbiol 56, 
2191–2197.

chemical grouping of aerobic actinomycetes. J Gen Appl Microbiol 29, 
319–322.

Table 1. Physiological characteristics of strain E71T and 
related type strains of Glycomyces species

<table>
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<th>Characteristic</th>
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<td>Acid produced from:</td>
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<td>D-Lactose</td>
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<td>D-Mannitol</td>
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<td>D-Melezitose</td>
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<td>D-Raffinose</td>
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<td>Inositol</td>
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<td>Methyl β-xylolide</td>
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<td>Assimilation of sole carbon sources:</td>
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<td>Hypoxanthine</td>
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<td>L-Tyrosine</td>
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Strains: 1, E71T; 2, G. algerensis NRRL B-16327T; 3, G. harbinensis 
IFO 14487T; 4, G. lechevalieri DSM 44724T; 5, G. rutgersensis IFO 
14488T. +, 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-xylitol and salicin, for decomposition of 
adename and casein, and for assimilation of benzoate. Data for reference 
strains were taken from Labeda et al. (1985) and Labeda & Kroppenstedt (2004).


