

# *Glycomyces salinus* sp. nov., an Actinomycete Isolated From a Hypersaline Habitat

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## Research Article

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## Abstract

A Gram-stain-positive, aerobic, non-motile actinobacterium, designated strain YIM 93776<sup>T</sup>, was isolated from a saline sediment sample collected from Aiding Lake in Xinjiang Uygur Autonomous Region, Northwest China. Phylogenetic analysis based on the 16S rRNA gene sequences showed that strain YIM 93776<sup>T</sup> was affiliated to the genus *Glycomyces*, and was closely related to *Glycomyces albus* TRM 49136<sup>T</sup> (97.6 % sequence similarity), *Glycomyces lacisalsi* XHU 5089<sup>T</sup> (97.0 %) and *Glycomyces anabasis* EGI 6500139<sup>T</sup> (96.2 %). The cell wall contained *meso*-diaminopimelic acid and the whole-cell hydrolysates sugars were galactose, mannose, arabinose, glucose and ribose. The predominant menaquinones were MK-9 (H<sub>4</sub>) and MK-10 (H<sub>4</sub>). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two phosphatidylglyceride, two unidentified phospholipids and two unidentified polar lipids were detected in the polar lipid extracts. Major fatty acids were anteiso-C<sub>17:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, anteiso-C<sub>15:0</sub> and anteiso C<sub>17:1</sub> A. The draft genome sequence of strain YIM 93776<sup>T</sup> was 5.37 Mbp in size with 69.5 mol% DNA G+C content. On the basis of morphological, chemotaxonomic and phylogenetic evidence, strain YIM 93776<sup>T</sup> therefore represents a novel species, for which the name *Glycomyces salinus* sp. nov. is proposed. The type strain is YIM 93776<sup>T</sup> (= KCTC 49430<sup>T</sup> = CGMCC 4.7685<sup>T</sup>).

## Introduction

The genus *Glycomyces* was initially proposed by Labeda et al. (1985) with *Glycomyces harbinensis* as the type species, and the description was later emended by Labeda and Kroppenstedt (2004). Strains belonging to the genus *Glycomyces* are Gram-stain-positive actinobacteria that form an extensively branched vegetative mycelium and aerial hyphae on certain growth media. These bacteria have a type II cell-wall composition (*meso*-diaminopimelic acid and glycine), whole-cell sugar pattern D (xylose, ribose, mannose, galactose and arabinose), and type PI phospholipid pattern with significant amounts of diphosphatidylglycerol. Most members of *Glycomyces* strains are isolated from soil, saline environments, and traditional Chinese medicinal plants tissues. At the time of writing, there are 26 validly published names in this genus (<https://lpsn.dsmz.de/genus/glycomyces>) with the latest described *Glycomyces terrestris* by Li et al. (2021), *Glycomyces albidus* by Qian et al. (2020), *Glycomyces buryatensis* by Nikitina et al. (2020), *Glycomyces xiaoerkulensis* by Wang et al. (2018), *Glycomyces sediminimaris* by Mohammadipanah et al. (2018), *Glycomyces paridis* by Fang et al. (2018), *Glycomyces dulcitolivorans* by Mu et al. (2018), and *Glycomyces anabasis* by Zhang et al. (2018). In the present study, we isolated a halophilic actinobacteria designated as strain YIM 93776<sup>T</sup> that was associated with genus *Glycomyces*. Based on the results of phylogenetic, chemotaxonomic and physiological characterization of strain YIM 93776<sup>T</sup>, we propose the strain YIM 93776<sup>T</sup> represents a novel *Glycomyces* species.

## Methods And Materials

### Bacterial isolation and cultivation

Strain YIM 93776<sup>T</sup> was isolated by using standard serial dilution plating technique from a hyper-saline sediment sample obtained from Aiding Lake (GPS, 42° 68'66"N, 89° 33'07"E) in Xinjiang Uygur Autonomous Region, Northwest China. The isolation plate (modified ISP 2 medium with 10 % (w/v) NaCl, Shirling and Gottlieb, 1966) were incubated at 37°C for 2 weeks. Presumptive actinomycete colonies were picked and re-streaked > 3 times to obtain axenic cultures. Strain YIM 93776<sup>T</sup> was selected among other strains for further characterization using polyphasic taxonomy based on the phylogenetic profiles of the 16S rRNA gene sequence. Pure cultures of strain YIM 93776<sup>T</sup> were routinely cultured and maintained on ISP 2 medium containing 10% (w/v) NaCl at 4°C and as glycerol suspensions (20%, v/v) at -80°C. Strain YIM 93776<sup>T</sup> was deposited in China General Microbiological Culture Collection Center (CGMCC) and Korean Collection for Type Cultures (KCTC) with the number CGMCC 4.7685<sup>T</sup> and KCTC 49430<sup>T</sup>, respectively.

### Phylogenetic and genomic analyses

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Feng et al. 2020). The almost-complete 16S rRNA gene sequence (1445 bp) was checked manually and submitted to the GenBank database. Pairwise similarity values between strain YIM 93776<sup>T</sup> and its closely related type strains were calculated via the EzBioCloud server (<https://www.ezbiocloud.net>) (Yoon et al. 2017). The 16S rRNA gene sequence was aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X (Thompson et al. 1997). Phylogenetic trees with gaps completely deleted were reconstructed based on the neighbour-joining (NJ) (Saitou et al. 1987), maximum-likelihood (ML) (Felsenstein et al. 1981) and maximum-parsimony (MP) (Fitch et al. 1971) algorithms, by using the MEGA version 7.0 software package (Kumar et al. 2016). Bootstrap analysis with 1000 replicates was applied to assess confidence levels of the branches (Felsenstein, 1985). Kimura's two-parameter model (Kimura, 1980) was used to calculate evolutionary distance matrices for the NJ methods. ML was calculated using the general time-reversible model with gamma-distribution with invariant sites (G + I).

The draft genome of strain YIM 93776<sup>T</sup> was sequenced using PacBio and Illumina Hiseq 2000 sequencers at Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). SOAPdenovo software (version 2.04) was employed to assemble paired-end reads (Li et al. 2010). The G + C contents (mole percent) were calculated from the genome sequences. To assess relationships between the strain YIM 93776<sup>T</sup> and sequenced related strains, we performed a phylogenomic analysis and constructed the evolutionary tree based on orthologous genes using the supermatrix method (Zhi et al. 2017).

### Phenotypic, physiological and biochemical characteristics

Cultural characteristics were determined after 7 and 14 days of incubation at 37 °C on different agar media (ISP 2, ISP 3, ISP 4 and ISP 5, Czapek's agar, PDA, and NA media) with 10 % (w/v) NaCl concentration. Growth at different temperatures (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 45, 50 and 55 °C) were determined on ISP 2 medium plates containing 10 % (w/v) NaCl concentration and observed after 7

and 14 days. Salt tolerance (0-30%, w/v, NaCl at 5% intervals) was examined at 37 °C on ISP 2 agar medium for 14 days. A series of pH conditions (4.0-11.0, at 0.5 intervals) using the buffer system described by Xu et al. (2005). Growth was assessed by monitoring turbidity as OD660 by using a spectroscopic method (Lambda 35 UV/Vis Spectrometer; PerkinElmer). After incubation on Czapek's agar (containing 10 %, w/v NaCl) at 37°C for 7 days and 28 days, morphological properties and spore motility were examined by means of a light microscope (DM2000, Leica) and a scanning electron microscope (XL30 ESEM-TMP, Philips-FEI). Gram staining was carried out by using the standard Gram reaction and was confirmed by using the KOH lysis test method. Carbon source utilization for growth was carried out on ISP 9 medium containing 10% (w/v) NaCl as described by Shirling and Gottlieb (1966). Nitrogen source utilization tests were carried out as described by Gordon et al. (1974). Catalase activity was detected based on bubble formation in 3 % (v/v) H<sub>2</sub>O<sub>2</sub> solution. Oxidase activity was detected by the oxidation of tetramethyl-p-phenylenediamine. Hydrolysis of starch, casein, gelatin, cellulose and Tweens 20, 40, and 80, nitrate reduction, urease activity, coagulation and peptonization of milk, melanin and H<sub>2</sub>S production were determined as described by Smibert and Krieg (1994). Other enzymatic activities of strain YIM 93776<sup>T</sup> were analyzed using API 20NE and API ZYM kits (bioMérieux, France) according to the manufacturer's instructions. The acid production from carbohydrates was determined using the API 50CH system (bioMérieux, France) according to the manufacturer's instructions.

### **Chemotaxonomic characteristics**

Biomass used for chemotaxonomic studies except fatty acid was obtained from cultures grown on ISP2 plate containing 10% (w/v) NaCl for 14 days at 37 °C . The diagnostic isomers of diaminopimelic acid were determined by HPLC according to methods used by Tang et al. (2009) and using TLC to verify it (Hasegawa et al.1983, Lechevalier et al.1970). The whole-cell sugar pattern and peptidoglycan amino acids were detected by HPLC according to methods used by Tang et al. (2009). The respiratory quinones were isolated using the method of Collins et al. (1977) and analysed by HPLC (Agilent Technologies 1260 Infinity) (Groth et al.1996). Polar lipids were extracted and then separated by using two dimensional TLC and identified using previously described procedures (Minnikin et al.1984; Hasegawa et al.1983). Molybdophosphoric acid, molybdenum blue, ninhydrin and  $\alpha$ -naphthol were used for the detection of total polar lipids, phospholipids, aminolipids and glycolipids, respectively. For cellular fatty acid analysis, strain YIM 93776<sup>T</sup> was grown on tryptic soya agar (TSA; Difco) with 10% (w/v) NaCl at 37°C and harvested after 7 days. Fatty acid methyl esters were extracted, methylated and analysed by using the Microbial Identification System (Sherlock version 6.1; MIDI database: TSBA6) according to the manufacturer's instructions (Sasser et al.1990).

## **Results And Discussion**

### **Molecular phylogenetic analysis**

16S rRNA gene sequence analysis showed that strain YIM 93776<sup>T</sup> was related most closely to the type strains of *Glycomyces albus* (97.6 % similarity), *Glycomyces lacisalsi* (97.0 % similarity), *Glycomyces*

*anabasis* (96.2 % similarity). Lower levels of 16S rRNA gene sequence similarity (92.0–95.1 %) were found with the type strains of all other type species within the genus *Glycomyces*. These values were below the 98.7 % cutoff point recommended for recognition of genomic species which need not to do DNA-DNA hybridization experiments (Jongsik et al. 2018). The neighbour-joining phylogenetic tree based on 16S rRNA gene sequences indicated strain YIM 93776<sup>T</sup> formed a branch distinct from those of their related species (Fig. 1). This relationship was supported by a high level of bootstrap support. The topologies of phylogenetic trees constructed with the maximum-likelihood and maximum-parsimony algorithms were similar to that of the tree reconstructed by neighbour-joining analysis (Supplementary information Fig. S1, S2). The draft genome of strain YIM 93776<sup>T</sup> is 5.36 Mbp long with a 69.5 mol% G + C content. The phylogeomic tree also supported that strain YIM 93776<sup>T</sup> formed a distinct phylogenetic lineage within the genus *Glycomyces* (Supplementary information Fig. S3). These results indicated that strain YIM 93776<sup>T</sup> should be considered as a separate novel species.

### Phenotypic, physiological and biochemical characteristics

Growth of strain YIM 93776<sup>T</sup> was observed at pH 5.5–11 (optimum 8) and in the presence of 5–13 % (w/v) NaCl. The temperature range for growth was 25–45°C, with optimum growth at 37°C. Strain YIM 93776<sup>T</sup> grew well on Czapek's agar and NA and moderately well on ISP 2, ISP 3, ISP 4, ISP 5 and PDA media (10 %, w/v NaCl). White substrate mycelia developed well on the above media. Aerial mycelium was white and abundantly produced on Czapek's and NA media, but absent on ISP 2, ISP 3, ISP 4 and ISP 5 media. Diffusible pigments or melanin were not observed on any test media. When strain YIM 93776<sup>T</sup> grown on Czapek's agar containing 10% (w/v) NaCl, fragmented substrate were observed on 7 days and aerial hyphae with fragmentation was detected on 28 days. The mobility of spores was not observed (Supplementary information Fig. S4). The detailed physiological characteristics of strain YIM 93776<sup>T</sup> are presented in Table 1, Table S1 and in the species description.

### Chemotaxonomic characteristics

Strains YIM 93776<sup>T</sup> contained meso-diaminopimelic acid as the cell-wall diamino acid, which was consistent with membership of the genus *Glycomyces*. The whole-cell sugar patterns of strain YIM 93776<sup>T</sup> contained galactose, mannose, arabinose, glucose and ribose, which differed slightly from reports for recognized species of the genus *Glycomyces* (Labeda et al. 2004) in that the novel strain did not contain xylose. The predominant menaquinones were MK-9 (H<sub>4</sub>) (50.3 %) and MK-10 (H<sub>4</sub>) (41.81 %). Major fatty acids (>10 %) of strain YIM 93776<sup>T</sup> were anteiso-C<sub>17:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, anteiso-C<sub>15:0</sub>, and anteiso C<sub>17:1</sub> A (Supplementary information Table S2). The polar lipids profiles of strain YIM 93776<sup>T</sup> were diphasatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two phosphatidylglyceride, two unidentified phospholipids and two unidentified polar lipids (Supplementary information Fig. S5).

The phylogenetic data indicate that strain YIM 93776<sup>T</sup> represents a novel species in the genus *Glycomyces*. In addition, the morphological and chemotaxonomic characteristics clearly differentiate

strain YIM 93776<sup>T</sup> from other related species of genus *Glycomyces* (Table 1). Strain YIM 93776<sup>T</sup> contained glucose, mannose, ribose, arabinose and galactose, but no xylose which *Glycomyces albus* does contain. In conclusion, on the basis of phylogenetic analysis, chemotaxonomic data and phenotypic traits, strain YIM 93776<sup>T</sup> is considered to represent a novel species in the genus *Glycomyces*, for which the name *Glycomyces salinus* sp. nov. is proposed.

### Description of *Glycomyces salinus* sp. nov.

*Glycomyces salinus* (sa.li'nus. N.L. masc. adj. *salinus* salted, saline)

Aerobic, Gram-stain-positive actinomycete. Aerial mycelium develops well on Czapek's and NA media. Soluble pigments are not produced. Aerial mycelium is long and fragmented with no branches. Aesculin, Tweens 20 and 40 were hydrolysed, but Tweens 80, starch and cellulose was not. Nitrate reduction, milk coagulation and peptonization, H<sub>2</sub>S production, gelatin liquefaction and urease are negative.

Temperature range for growth is 25–45°C with the optimum at 37°C; pH range for growth is pH 5.5–11.0 and the optimum is pH 8; NaCl tolerance range for growth is 5–13 % (w/v) NaCl and the optimum is 10 % (w/v) NaCl. *D*-mannitol, maltitol, maltose,  $\alpha$ -lactose, fructose and  $\alpha$ -*D*-glucose are utilized as sole carbon sources. *L*-phenylalanine, adenine, glycine, *L*-methionine, *L*-threonine, aspartic acid, *L*-lysine, arginine, *L*-tyrosine, *L*-asparagine and alanine are utilized as sole nitrogen sources. Results from API 50CH tests showed that acids produced from erythritol, *D*-arabinose, *L*-arabinose, *D*-ribose, *D*-xylose, *D*-galactose, *D*-glucose, fructose, *D*-mannose, *L*-rhamnose, inositol, arbutin, esculin ferric citrate, salicin, *D*-cellobiose, *D*-maltose, *D*-saccharose, *D*-trehalose, *D*-melezitose, Amidon, glycogen, *D*-tagatose, *D*-fucose, *L*-fucose, potassium 5-ketogluconate. For enzyme activities (API ZYM system), it is positive for  $\alpha$ -glucosidase, naphthol-AS-BI-phosphohydrolase. Contains *meso*-diaminopimelic acid as the diamino acid. The whole-cell sugar pattern consists of galactose, mannose, arabinose, glucose and ribose. The polar lipids pattern consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two phosphatidylglyceride, two unidentified phospholipids and two unidentified polar. The predominant menaquinones are MK-9 (H<sub>4</sub>) and MK-10 (H<sub>4</sub>). Major cellular fatty acids are anteiso-C<sub>17:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, anteiso-C<sub>15:0</sub>, and anteiso C<sub>17:1</sub> A.

The type strain, YIM 93776<sup>T</sup> (KCTC 49430<sup>T</sup> = CGMCC 4.7685<sup>T</sup>), was isolated from a hyper-saline sediment sample obtained from Aiding Lake in Xinjiang Uygur Autonomous Region, Northwest China. The DNA G + C content of the type strain is 69.5 mol%.

## Abbreviations

CA, Czapek's agar; PDA, potato dextrose agar; NA, nutrient agar media; ISP 3, Oat agar; ISP 5, glycerol/asparagine agar; ISP 4, inorganic salts-starch agar; ISP2, yeast extract-malt extract agar; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, Phosphatidylglyceride; UL, unidentified polar lipids; PI, phosphatidylinositol; PL, unidentified phospholipids

The NCBI GenBank accession number for the 16S rRNA gene sequence of strain YIM 93776<sup>T</sup> is MW380665. The draft whole genome sequence for strain YIM 93776<sup>T</sup> has been deposited at DDBJ/ENA/GenBank under accession number GCA\_016428645.1.

## Declarations

### Author contributions

RL and E-MZ carried out the data analysis and wrote the manuscript. RL, YW and Y-GC performed the experiments. G-QJ provided the samples. E-MZ and S-KT supervised the project. All the authors discussed the results and contributed to the final manuscript.

### Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Table

Table 1

Differential characteristics of strain YIM 93776<sup>T</sup> and other phylogenetically closely related species.

Characteristic	1	2	3	4
Growth:				
Temperature range (optimum, °C)	25–45 (37)	25–40 (30)	20–50 (30–35)	15–40 (30)
NaCl range (optimum, %)	5–13 (10)	0–13 (5–7)	0–9 (1–3)	0–10 (4–6)
pH range (optimum)	5.5–11 (8)	6–14 (8–9)	6–12 (7–8)	6–10 (7–8)
Nitrate reduction	-	+	-#	-
Urease	-	+	-#	NR
Oxidase	-	+	-	+
Hydrolysis of:				
Starch	-	+	-	+
Cellulose	-	+	+	+
Tweens 80	-	+	+	+
Utilization of:				
Arabinose	-	+	-	-
Xylose	-	+	-	-
Raffinose	-	+	-	-
D-galactose	-	+	-	+
Maltose	+	+	-	NR
Sucrose	-	-	+	NR
Maltitol	+	-	-	NR
α-lactose	+	-	-	NR
α-D-glucose	+	-	+	-
Cellobiose	-	-	+	-
D-fructose	-	+	-	+

Characteristic	1	2	3	4
Glycerol	-	+	-	-
Inositol	-	+	+	-
D-mannose	-	-	+	-
Melibiose	-	-	+	-
L-rhamnose	-	+	-	-
D-ribose	-	+	-	-
D-sorbitol	-	+	-	-
Whole-cell sugars	galactose, mannose, arabinose, glucose, ribose	xylose, ribose, arabinose	glucose, galactose	glucose, mannose, ribose, galactose, xylose
Polar lipids	DPG, PL, PGL, PG, PI, UL	PG, DPG, PI, PIM, GL, PGL	DPG, PG, PI, PL, GL <sup>#</sup>	PG, DPG, PI, PIM, GL, PL, ULs
Major menaquinones	MK-9(H <sub>4</sub> ), MK-10(H <sub>4</sub> )	MK-9(H <sub>4</sub> ), MK-9(H <sub>6</sub> )	MK-10(H <sub>4</sub> ), MK-9(H <sub>4</sub> ), MK-10(H <sub>2</sub> ), MK-11	MK-11, MK-11(H <sub>2</sub> )
Major fatty acids (> 10 %)	anteiso-C <sub>17:0</sub> , iso-C <sub>15:0</sub> , iso-C <sub>16:0</sub> , anteiso-C <sub>15:0</sub> , anteiso C <sub>17:1</sub> A	anteiso-C <sub>17:0</sub> , anteiso-C <sub>15:0</sub> , iso-C <sub>15:0</sub> , iso-C <sub>16:0</sub>	anteiso-C <sub>17:0</sub> , anteiso-C <sub>15:0</sub> , iso-C <sub>15:0</sub> <sup>#</sup>	anteiso-C <sub>17:0</sub> , anteiso-C <sub>15:0</sub> , iso-C <sub>15:0</sub> , iso-C <sub>16:0</sub>
DNA G + C content (mol%)	69.5	71.0	68.6	70.4

<sup>#</sup>Data was confirmed in this work.

Taxa:1. Strain YIM 93776<sup>T</sup>; 2. *G. albus* TRM 49136<sup>T</sup>; (3) *G. lacisalsi* XHU 5089<sup>T</sup>; (4) *G. anabasis* EGI 6500139<sup>T</sup>. Data for strain YIM 93776<sup>T</sup> was taken from this study. Data for *G. albus* TRM 49136<sup>T</sup>, *G. lacisalsi* XHU 5089<sup>T</sup>, and *G. anabasis* EGI 6500139<sup>T</sup> was taken from Han *et al.* (2014), Guan *et al.* (2016), and Zhang *et al.* (2018), respectively, unless otherwise specified. +, Positive; -, negative; NR: not reported. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PGL, Phosphatidylglyceride; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PL, phospholipids; GL, unidentified glycolipid; PIM, phosphatidylinositol mannoside; UL, unidentified polar lipid.

## Figures

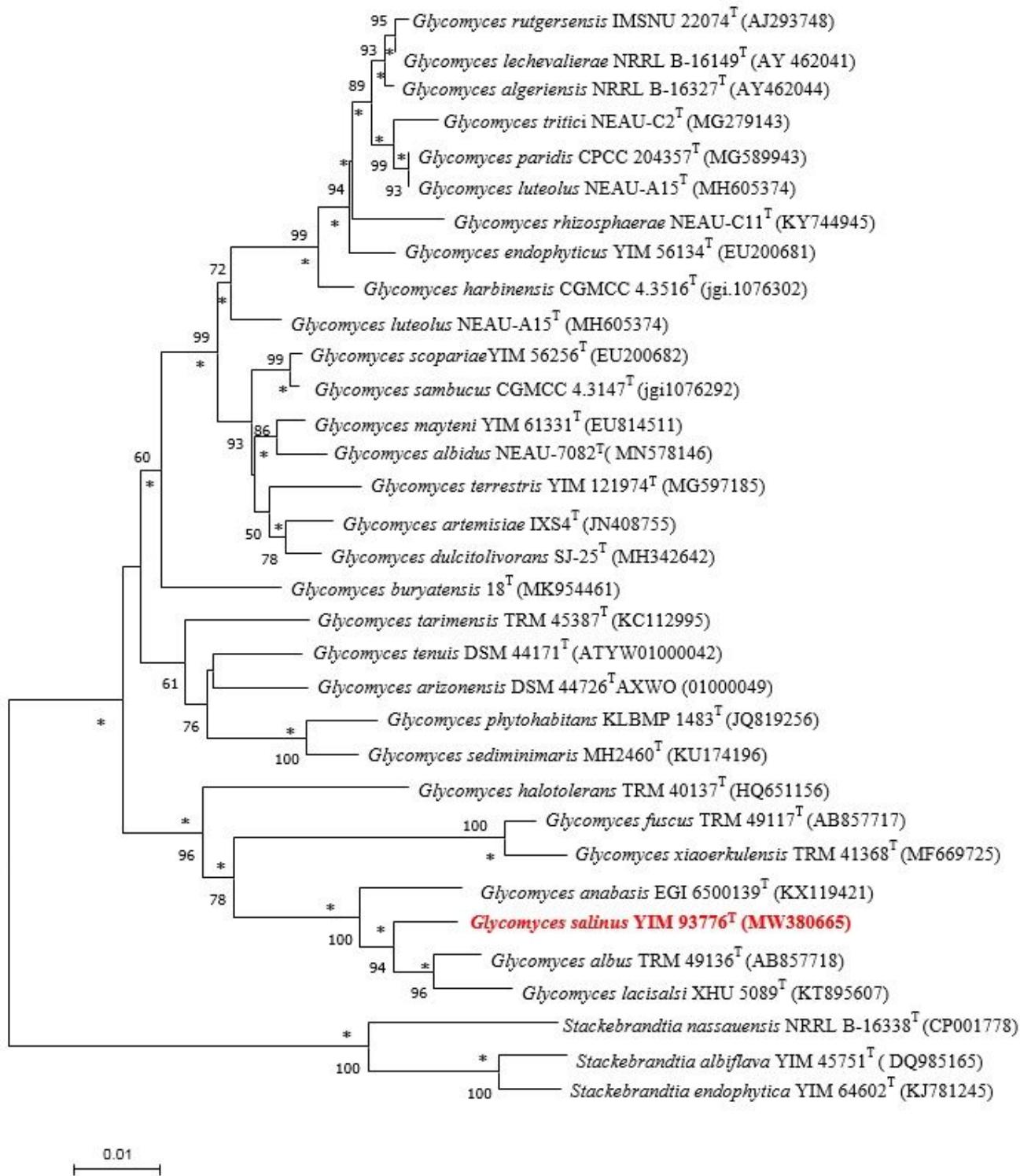


Figure 1

Neighbour-joining tree based on 16S rRNA gene sequences showing the position of strain YIM 93776T within the genus *Glycomyces*. Sequences of *Stackebrandtia nassauensis* NRRL B-16338T, *Stackebrandtia albiflava* YIM 45751T and *Stackebrandtia endophytica* YIM 64602T were used as outgroups. Bootstrap values (based on 1000 replications) greater than 50 % are given at branching

points. Asterisks denote nodes that were also recovered using the maximum-likelihood and maximum-parsimony methods. Bar: one substitution per 100 nucleotide position.

## Supplementary Files

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- [Supplementarymaterials.docx](#)