Haloglycomyces albus gen. nov., sp. nov., a halophilic, filamentous actinomycete of the family *Glycomycetaceae*

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A novel halophilic actinobacterium, designated YIM 92370^T, was isolated from a hypersaline habitat in Xinjiang Province, north-west China. The strain was aerobic, Gram-positive-staining and halophilic, with an optimum NaCl concentration for growth of 8–12 % (w/v). The whole-cell sugar pattern consisted of ribose, xylose and glucose. The predominant menaquinone was MK-9(H₄) and the major fatty acids were iso-C_{16:0}, iso-C_{17:0} and anteiso-C_{17:0}. The phospholipid pattern consisted of phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, two unknown phosphoglycolipids and one unknown phospholipid. The G + C content of the genomic DNA was 60.8 mol%. Phylogenetic analysis showed that strain YIM 92370^T can be distinguished from representatives of *Glycomyces* and *Stackebrandtia*, the two existing genera in the family *Glycomycetaceae*, by low 16S rRNA gene sequence similarities (<93.7 %). Strain YIM 92370^T therefore represents a novel genus and species of the family *Glycomycetaceae*, for which the name *Haloglycomyces albus* gen. nov., sp. nov. is proposed. The type strain of *Haloglycomyces albus* is YIM 92370^T (=DSM 45210^T =KCTC 19481^T).

The family *Glycomycetaceae*, which belongs to the suborder *Glycomycineae* (Stackebrandt *et al.*, 1997), currently comprises the genera *Glycomyces* and *Stackebrandtia*. The genus *Glycomyces* was originally established by Labeda *et al.* (1985) and its description was later emended by Labeda & Kroppenstedt (2004). The genus *Glycomyces* is the type genus of the family *Glycomycetaceae* and consists of eight species with validly published names, *Glycomyces algeriensis*, *G. arizonensis*, *G. endophyticus*, *G. harbinensis*, *G. lechevalierae*, *G. rutgersensis*, *G. sambucus* and *G. tenuis* (Labeda *et al.*, 1985; Evtushenko *et al.*, 1991; Labeda & Kroppenstedt, 2004; Gu *et al.*, 2007; Qin *et al.*, 2008). The genus *Stackebrandtia* (Labeda & Kroppenstedt, 2005) contains the single species *Stackebrandtia nassauensis*. To date, there have been no reports of halophilic strains in the family *Glycomycetaceae*.

During an investigation of actinomycete diversity in hypersaline habitats, strain YIM 92370^T was isolated from a soil sample collected from Xinjiang Province, north-west China, by using cellulose-casein medium with multi-salts as described by Tang *et al.* (2008). The aim of this study was to identify the exact taxonomic status of strain YIM 92370^T by using a polyphasic taxonomy study.

Strain YIM 92370^T was grown and observed on media (ISP2, ISP3, ISP4, ISP5, nutrient agar and Czapek's agar plates with 10 % NaCl) commonly used for characterization of *Streptomyces* species (Shirling & Gottlieb, 1966) at 37 °C for 2–4 weeks. The colony colour was determined with the ISCC–NBS colour charts (Kelly, 1964). Morphological characteristics of mycelia were examined by light microscopy (Olympus BH-2) and scanning electron microscopy (JSM 5600LV; JEOL) of 32-day-old cultures grown on ISP4 medium supplemented with 10 % NaCl. Growth was tested over ranges of temperatures (4–55 °C) and pH (pH 4.0–10.0) as described by Xu *et al.* (2005) on ISP4 medium supplemented with 10 % NaCl. Tolerance of different NaCl concentrations (0–25 %, w/v) was tested on ISP4 as the basal medium. The other

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 92370^T is EU660053.

Profiles of cell-wall sugars and polar lipids of strain YIM 92370^T are available as supplementary material with the online version of this paper.

physiological characteristics of strain YIM 92370^T, including utilization of sole carbon sources for energy and growth, were assessed by using the media and methods of Gordon *et al.* (1974). All the media were supplemented with 10 % NaCl.

Scanning electron micrographs of well-developed aerial mycelium and substrate mycelium are shown in Fig. 1. The colonies were white on most media tested (ISP4, ISP2, Czapek's and nutrient agar), but aerial mycelium did not develop on ISP3 or ISP5 media. No diffusible pigment was produced on any of the media tested. The optimal temperature and pH for growth were 37 °C and pH 7.0–7.5. The NaCl concentration for growth was 3–18 %, with optimal growth at 8–12 %. The other physiological characteristics of strain YIM 92370^T are given in the genus and species descriptions.

Standard procedures were used to determine the cell-wall amino acids and sugars (Hasegawa *et al.*, 1983). Menaquinones were extracted using the method of Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1997). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al.* (1984). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.). Chemo-

taxonomic data supported the separation of strain YIM 92370^T from the genera *Glycomyces* and *Stackebrandtia*, although all three genera have *meso*-diaminopimelic acid as the diagnostic amino acid. The whole-cell sugar pattern for strain YIM 92370^T consists of ribose, xylose and glucose (Supplementary Fig. S1, available in IJSEM Online) whereas, in all known strains of *Glycomyces* and *Stackebrandtia*, the diagnostic sugars are ribose, xylose, mannose, galactose and inositol. Strain YIM 92370^T and strains of the genera *Stackebrandtia* and *Glycomyces* contain phosphatidylglycerol and diphosphatidylglycerol, but there are also some differences between their polar lipid profiles. For example, in comparison with strains of the genus *Glycomyces*, YIM 92370^T exhibits an additional unknown phospholipid while, in comparison with the type strain of the type species of the genus *Stackebrandtia*, YIM 92370^T also contains phosphatidylinositol, phosphatidylinositol mannosides and two unknown phosphoglycerolipids (Supplementary Fig. S2). The menaquinone pattern of strain YIM 92370^T, which consists of MK-9(H₄), MK-9(H₂), MK-8(H₄), MK-10(H₄) and MK-10(H₂), is different from those of members of the genera *Glycomyces* and *Stackebrandtia* (Table 1). The pattern of major fatty acids, consisting of iso-C_{16:0} (15.04 %), iso-C_{17:0} (10.35 %) and anteiso-C_{17:0} (51.92 %), clearly separates the new isolate YIM 92370^T from the genera *Glycomyces* and *Stackebrandtia* (Table 2).

Amplification and sequencing of the 16S rRNA gene were performed as described by Cui *et al.* (2001). The almost-

Fig. 1. Scanning electron micrographs of strain YIM 92370^T grown on ISP4 medium supplemented with 10 % NaCl for 21 days at 37 °C. Bars, 2 µm.

Table 1. Menaquinone profiles of strain YIM 92370^T and type strains of related species in the family *Glycomycetaceae*

Strains: 1, *Haloglycomyces albus* gen. nov., sp. nov. YIM 92370^T; 2, *S. nassauensis* NRRL B-16338^T; 3, *G. algeriensis* NRRL B-16327^T; 4, *G. arizonensis* NRRL B-16153^T; 5, *G. harbinensis* NRRL 15337^T; 6, *G. lechevalierae* NRRL B-16149^T; 7, *G. rutgersensis* NRRL B-16106^T; 8, *G. tenuis* NRRL B-16895^T; 9, *G. sambucus* DSM 45047^T. Values are percentages of total menaquinones. +, Present; ±, minor component; –, not present or not reported; tr, trace amount. Data for reference strains were taken from Labeda & Kroppenstedt (2005) and Gu *et al.* (2007).

Menaquinone	1	2	3	4	5	6	7	8	9
MK-8(H ₄)	13	–	–	–	–	–	–	–	–
MK-9(H ₂)	27	–	–	–	–	–	–	–	–
MK-9(H ₄)	42	–	–	–	–	–	–	±	–
MK-10	–	–	23	–	–	17	–	–	–
MK-10(H ₂)	5	–	–	18	+	21	+	–	–
MK-10(H ₄)	10	30	–	52	–	11	–	–	–
MK-10(H ₆)	–	16	–	tr	+	–	±	+	–
MK-11	–	–	65	–	–	24	–	–	69
MK-11(H ₂)	–	–	–	9	–	19	–	–	–
MK-11(H ₄)	–	31	–	22	–	8	–	–	26
MK-11(H ₆)	–	23	–	–	–	–	–	+	–
MK-12	–	–	12	–	–	tr	–	–	–

Table 2. Comparison of the fatty acid profiles of type strains of the genera *Haloglycomyces* gen. nov., *Stackebrandtia* and *Glycomyces*

Strains: 1, *Haloglycomyces albus* YIM 92370^T; 2, *S. nassauensis* NRRL B-16338^T; 3, *G. algeriensis* NRRL B-16327^T; 4, *G. arizonensis* NRRL B-16153^T; 5, *G. harbinensis* NRRL 15337^T; 6, *G. lechevalierae* NRRL B-16149^T; 7, *G. rutgersensis* NRRL B-16106^T; 8, *G. tenuis* NRRL B-16895^T; 9, *G. sambucus* DSM 45047^T. Data for reference strains were taken from Labeda & Kroppenstedt (2005), Gu *et al.* (2007) and Qin *et al.* (2008). Values are percentages of total fatty acids; –, not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8	9
14:0 iso	0.58	–	2.04	3.13	1.51	2.86	2.37	2.21	7.20
14:0	0.15	–	–	–	0.49	0.45	0.61	–	–
15:0 iso	7.12	8.70	14.41	7.18	11.26	14.77	19.90	13.95	8.20
15:0 anteiso	7.25	2.89	27.52	25.36	15.11	32.52	30.95	33.20	26.20
15:1	–	0.84	–	–	–	–	–	–	–
15:0	0.84	0.64	–	–	0.41	2.01	2.98	2.07	–
16:1 iso	0.29	2.11	4.37	11.53	10.34	4.14	4.37	2.59	5.40
16:0 iso	15.04	8.72	10.90	22.69	17.83	16.19	12.52	16.00	28.80
16:1 <i>cis</i> 7	–	0.53	–	–	–	–	–	–	–
16:1 <i>cis</i> 9	–	3.17	–	–	0.62	–	–	–	–
16:0	3.29	0.67	–	1.34	2.03	1.31	1.61	1.92	–
16:0 10-methyl	–	9.07	–	–	–	–	–	–	–
17:1 iso ω 9c	0.33	–	–	–	–	–	–	–	–
17:1 iso	–	0.58	–	–	–	–	–	–	–
17:1 anteiso	–	1.70	12.33	8.75	12.66	5.15	6.13	3.62	2.80
17:0 iso	10.35	9.02	2.85	1.01	1.34	1.36	0.98	1.58	1.40
17:0 anteiso	51.92	26.86	25.59	19.01	26.41	19.24	16.82	22.86	18.00
17:1 <i>cis</i> 9	–	1.84	–	–	–	–	0.42	–	–
17:0	1.15	–	–	–	–	–	0.34	–	–
17:0 10-methyl	–	1.44	–	–	–	–	–	–	–
16:0 2-OH	–	4.22	–	–	–	–	–	–	–
17:0 2-OH	–	2.45	–	–	–	–	–	–	–
17:0 anteiso 2-OH	–	14.52	–	–	–	–	–	–	–
18:0 iso	0.38	–	–	–	–	–	–	–	–
19:0 anteiso	0.26	–	–	–	–	–	–	–	–

complete 16S rRNA gene sequence of strain YIM 92370^T (1421 nt) was obtained and compared with available 16S rRNA gene sequences in GenBank/EMBL/DDBJ using BLAST searches (Altschul *et al.*, 1990). The sequence of strain YIM 92370^T was manually aligned with 16S rRNA gene sequences of all recognized species in the family *Glycomycetaceae*. Phylogenetic analysis was performed using the software package MEGA version 3.1 (Kumar *et al.*, 2004). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. Evolutionary distance matrices were generated as described by Kimura (1980). The robustness of the tree topologies was assessed by bootstrap analyses based on 1000 replications (Felsenstein, 1985). The DNA G+C content of strain YIM 92370^T (60.8 mol%) was determined by using the HPLC method (Mesbah *et al.*, 1989).

Phylogenetic analysis demonstrated clearly that strain YIM 92370^T belongs to the family *Glycomycetaceae* (Stackebrandt *et al.*, 1997) and is very well separated from all *Glycomyces* and *Stackebrandtia* type strains, which

represent the nearest phylogenetic neighbours. The 16S rRNA gene sequence of strain YIM 92370^T showed 93.7% similarity to that of *G. arizonensis* NRRL B-16153^T and 82.8–93.5% to other strains of the family *Glycomycetaceae*. Although strain YIM 92370^T was closely related to species of the genus *Glycomyces* on the basis of 16S rRNA gene sequence similarity, the novel strain was not included in the clades of the genera *Glycomyces* or *Stackebrandtia* (Fig. 2). Therefore, phylogenetically, strain YIM 92370^T should be recognized as representing a distinct genus in the family *Glycomycetaceae*.

In conclusion, phylogenetic analyses based on 16S rRNA gene sequences, chemotaxonomic data and phenotypic traits indicated that strain YIM 92370^T should be classified in a novel genus and species, for which the name *Haloglycomyces albus* gen. nov., sp. nov. is proposed.

Description of *Haloglycomyces* gen. nov.

Haloglycomyces [Ha'lo.gly.co.my'ces. Gr. n. *hals* salt; N.L. masc. n. *Glycomyces* a bacterial genus name; N.L. masc. n. *Haloglycomyces* a salt-(loving) *Glycomyces*-like bacterium].

Fig. 2. Phylogenetic tree of strain YIM 92370^T and its nearest neighbours calculated from 16S rRNA gene sequences using Kimura's evolutionary distance method (Kimura, 1980) and the neighbour-joining method of Saitou & Nei (1987). Bar, 0.01 nucleotide substitutions per site.

Aerobic, Gram-positive, halophilic and filamentous actinomycetes. Aerial mycelium develops well on most media tested and branched substrate mycelium fragments into short or elongated rods (approx 0.45 mm in diameter). Oxidase-negative and catalase-positive. Contain *meso*-diaminopimelic acid as the diamino acid. The whole-cell sugar pattern consists of ribose, xylose and glucose. The menaquinones are MK-9(H₄), MK-9(H₂), MK-8(H₄), MK-10(H₄) and MK-10(H₂). The polar lipid pattern consists of phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, two unknown phosphoglycolipids and one additional unknown phospholipid. Major cellular fatty acids are iso-C_{16:0}, iso-C_{17:0} and anteiso-C_{17:0}. The DNA G+C content of the type strain of the type species is 60.8 mol% (HPLC). Phylogenetically, the genus is a member of the family *Glycomycetaceae*. The 16S rRNA signature nucleotides are 126:235 (A-U), 137:226 (U-G), 146:176 (G-U), 379:384 (G-C), 407:435 (G-C), 408:434 (A-U), 418:425 (U-A), 501:544 (U-G), 615:625 (U-A), 617:623 (A-U), 896:903 (U-G), 1124:1149 (A-U), 1165:1171 (U-A), 1263:1272 (G-U) and 1265:1270 (U-G). The type species is *Haloglycomyces albus*.

Description of *Haloglycomyces albus* sp. nov.

Haloglycomyces albus (al'bus, L. masc. adj. *albus* white, referring to the white aerial mycelium).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. Substrate mycelium is yellow-white to deep orange-yellow on tested media. White aerial hyphae are produced on ISP2, ISP4, nutrient agar and Czapek's agar plates with 10% NaCl. No

diffusible pigment is produced. Temperature and pH ranges for growth are 15–40 °C and pH 5.0–9.0, with optima at 37 °C and pH 7.0–7.5. No growth at 10 or 45 °C or above pH 9.0. Growth occurs in the presence of 3–18% NaCl, but not below 3% NaCl; optimal concentration 8–12% NaCl. Utilizes cellobiose, glucose, maltose, mannitol, mannose, rhamnose and sorbitol as sole carbon sources, but not D-arabinose, dextrin, fructose, galactose, glycerol, inositol, lactose, melibiose, raffinose, ribose, xylitol or xylose. Positive for gelatin hydrolysis, starch hydrolysis, milk coagulation and peptonization, but negative for urease, cellulose hydrolysis, production of H₂S and nitrate reduction.

The type strain, YIM 92370^T (=DSM 45210^T=KCTC 19481^T), was isolated from a soil sample from a hypersaline habitat in Xinjiang Province, north-west China.

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