Stackebrandtia nassauensis gen. nov., sp. nov. and emended description of the family *Glycomycetaceae*

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During the course of a 16S rRNA gene sequence phylogenetic evaluation of putative *Glycomyces* strains, it was noted that strain NRRL B-16338^T is phylogenetically nearest to the genus *Glycomyces* but apparently is not a member of this or any of the other currently described actinomycete genera. The strain was subjected to a polyphasic study using standard methods for chemotaxonomic, morphological and physiological evaluation. The strain exhibited chemotaxonomic characteristics distinct from *Glycomyces* in spite of having 16S rRNA gene sequence similarity of 92 % with the described species of this genus. The whole-cell sugar pattern of NRRL B-16338^T consisted of ribose and inositol, with traces of arabinose and mannose. The phospholipids observed were phosphatidylglycerol and diphosphatidylglycerol and menaquinones consisting of MK-10(H₄), MK-10(H₆), MK-11(H₄) and MK-11(H₆). A significant quantity (14.5 %) of 17 : 0 anteiso 2-hydroxy fatty acid was observed in the fatty acid profile of this strain. These characteristics clearly differentiate NRRL B-16338^T from members of the genus *Glycomyces* and it is proposed that the strain represents a new genus within the family *Glycomycetaceae* to be called *Stackebrandtia* gen. nov. The description of this family is emended to permit its inclusion. It is proposed that the type species of the genus should be named *Stackebrandtia nassauensis*. The type strain LLR-40K-21^T (= NRRL B-16338^T = DSM 44728^T) was isolated from a soil sample from Nassau, Bahamas.

During the course of a phylogenetic evaluation of putative strains of *Glycomyces* based on 16S rRNA gene sequences (Labeda & Kroppenstedt, 2004), strain LLR-40K-21^T (= NRRL B-16338^T = DSM 44728^T), isolated by Mary P. Lechevalier from a soil sample collected on the road side in Nassau, Bahamas, was found to be phylogenetically most closely related to the genus *Glycomyces*, but clearly not a member this genus. The strain was subjected to a polyphasic study using standard methods for chemotaxonomic and morphological evaluation. In addition to strain NRRL B-16338^T, the type strains of all described *Glycomyces* species were included in this study, i.e. *Glycomyces rutgersensis* NRRL B-16106^T, *G. harbinensis* NRRL 15337^T, *G. tenuis* NRRL B-16895^T, *G. algeriensis* NRRL B-16327^T, *G.*

arizonensis NRRL B-16153^T and *G. lechevalieriae* NRRL B-16149^T.

The strain was cultivated on NZ-amine starch agar (DSMZ medium no. 554) at 28 °C. Morphological observations were made on yeast extract-malt extract agar (ISP-2), inorganic salts-starch agar (ISP-4), glycerol-asparagine agar (ISP-5) (Shirling & Gottlieb, 1966), Czapek's sucrose agar (Pridham & Lyons, 1980) and DSMZ medium no. 554 (DSMZ, 2001).

For the analyses of fatty acids, about 40 mg of cells was scraped from agar plates. For the other chemical analyses, cells were grown in liquid medium and harvested by centrifugation. Chemotaxonomic analyses of strains for polar lipids, menaquinones and fatty acids were performed using previously described methods (Grund & Kroppenstedt, 1989; Minnikin *et al.*, 1984; Sasser, 1990). Cell-wall diamino acid isomer was determined by the method of Stanek & Roberts (1974) and whole-cell sugar content was determined as alditol acetates by the method of Saddler *et al.* (1991). The acyl type of the muramic acid in the cell wall was determined by the method of Uchida & Aida (1977).

Published online ahead of print on 1 April 2005 as DOI 10.1099/ij.s.0.63496-0.

Abbreviation: DAP, 2,6-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Stackebrandtia nassauensis* sp. nov. NRRL B-16338^T is AY650268.

Genomic DNA for sequencing of the 16S rRNA gene was isolated from growth on DSMZ medium no. 554 plates using UltraClean microbial DNA isolation kits (Mo Bio Laboratories) and then amplified and sequenced following previously described procedures (Labeda & Kroppenstedt, 2000). The 16S rRNA gene sequence was deposited in GenBank with the accession number AY650268. A phylogenetic tree was constructed according to the neighbour-joining method of Saitou & Nei (1987) and the stability of the groupings was estimated by bootstrap analysis (Felsenstein, 1989). The G+C content of the DNA was determined by HPLC according to Mesbah *et al.* (1989).

Physiological tests, including production of acid from carbohydrates, utilization of organic acids and hydrolysis and decomposition of adenine, guanine, hypoxanthine, tyrosine, xanthine, casein, aesculin, urea and hippurate, were evaluated using the media of Gordon *et al.* (1974). Phosphatase activity was measured using the method of Kurup & Schmitt (1973). The temperature range for growth was determined on slants of DSMZ Medium No. 554.

Phylogenetic analysis clearly demonstrated that strain NRRL B-16338^T belongs to the order *Actinomycetales* (Stackebrandt *et al.*, 1997) and is well separated from all *Glycomyces* type strains (16S rRNA gene sequence similarity to the *Glycomyces* species studied is only 92%) that represent the nearest phylogenetic neighbours (Fig. 1).

Evaluation of the data with several other phylogenetic algorithms, including maximum-parsimony and maximum-likelihood (Felsenstein, 1989), provided dendrograms that exhibited a stable relationship between NRRL B-16338^T and the species within the genus *Glycomyces*. The exact placement of the suborder *Glycomycineae* within the class *Actinobacteria* is somewhat different for each computational method, possibly reflecting the small number of taxa available and sequenced within this suborder.

Chemotaxonomic data supported the separation of strain NRRL B-16338^T from the genus *Glycomyces*, as can be seen in Table 1. The whole-cell sugar pattern for strain NRRL B-16338^T consists of ribose and inositol, with traces of arabinose and mannose, whereas in all *Glycomyces* strains, ribose, xylose mannose and galactose are found as diagnostic sugars. NRRL B-16338^T and all of the *Glycomyces* type strains were found to contain *N*-glycolylmuramic acid. Strain NRRL B-16338^T and the *Glycomyces* strains synthesize phosphatidylglycerol and diphosphatidylglycerol but NRRL B-16338^T exhibits two additional unknown phospholipids; all *Glycomyces* strains are rich in sugar-containing phospholipids such as phosphatidylinositolmannosides and two phosphoglycolipids of unknown structure. The fatty acid pattern clearly separates NRRL B-16338^T from the *Glycomyces* strains by the occurrence of 10-methyl-branched heptadecanoic acid and iso-branched 2-hydroxy fatty acids which are missing in *Glycomyces* (Table 2). The

Fig. 1. Phylogenetic tree of the proposed genus *Stackebrandtia* and near 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.

Table 1. Comparison of chemotaxonomic characteristics of *Stackebrandtia* and *Glycomyces*

Abbreviations: PI, Phosphatidylinositol; PIM, phosphatidylinositolmannosides; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PGL_{1,2}, unknown phosphoglycolipids; PL_{1,2}, unknown phospholipids.

	DAP isomer	Whole-cell sugars	Polar lipids
<i>Stackebrandtia</i>	meso-DAP	Inositol, ribose	PG, DPG, PL ₁ , PL ₂
<i>Glycomyces</i>	meso-DAP	Galactose, mannose, ribose, xylose	PIM, PI, PG, DPG, PGL ₁ , PGL ₂

menaquinone pattern of NRRL B-16338^T, where the predominant menaquinones are MK-10(H₄), MK-10(H₆), MK-11(H₄) and MK-11(H₆), is also different from that of the genus *Glycomyces*, where the pattern appears almost species-specific but consists of menaquinones with 9, 10, 11 or 12 isoprene units with various degrees of hydrogenation (Table 3).

The gross morphological characteristics of NRRL B-16338^T are stated in the description below. Fragmentation of aerial or substrate mycelium into spore-like bodies has not been observed on any of the media studied. Moreover,

observations of wet mounts of substrate and aerial mycelium from various media by phase-contrast light microscopy have never revealed any motile spores. Aerial mycelium has been observed twisting into a spiral morphology on yeast extract-malt extract agar (Fig. 2), while observations of growth on Czapek's sucrose agar (not shown) have revealed regions of aerial mycelia that appear almost ready to fragment into spores, although the latter process has never been found.

The physiological properties of the strain are summarized in the formal species description below.

Table 2. Fatty acid profiles of *Stackebrandtia* and *Glycomyces*

Species: 1, *Stackebrandtia nassauensis* NRRL B-16338^T; 2, *Glycomyces algeriensis* NRRL B-16327^T; 3, *G. arizonensis* NRRL B-16153^T; 4, *G. harbinensis* NRRL 15337^T; 5, *G. lechevalierae* NRRL B-16149^T; 6, *G. rutgersensis* NRRL B-16106^T; 7, *G. tenuis* NRRL B-16895^T.

Fatty acid	1	2	3	4	5	6	7
14:0 iso	—	2.04	3.13	1.51	2.86	2.37	2.21
14:0	—	—	—	0.49	0.45	0.61	—
15:0 iso	8.70	14.41	7.18	11.26	14.77	19.90	13.95
15:0 anteiso	2.89	27.52	25.36	15.11	32.52	30.95	33.20
15:1	0.84	—	—	—	—	—	—
15:0	0.64	—	—	0.41	2.01	2.98	2.07
16:1 iso	2.11	4.37	11.53	10.34	4.14	4.37	2.59
16:0 iso	8.72	10.90	22.69	17.83	16.19	12.52	16.00
16:1 <i>cis</i> 7	0.53	—	—	—	—	—	—
16:1 <i>cis</i> 9	3.17	—	—	0.62	—	—	—
16:0	0.67	—	1.34	2.03	1.31	1.61	1.92
16:0 10-methyl	9.07	—	—	—	—	—	—
17:1 iso	0.58	—	—	—	—	—	—
17:1 anteiso	1.70	12.33	8.75	12.66	5.15	6.13	3.62
17:0 iso	9.02	2.85	1.01	1.34	1.36	0.98	1.58
17:0 anteiso	26.86	25.59	19.01	26.41	19.24	16.82	22.86
17:1 <i>cis</i> 9	1.84	—	—	—	—	0.42	—
17:0	—	—	—	—	—	0.34	—
17:0 10-methyl	1.44	—	—	—	—	—	—
16:0 2-hydroxy	4.22	—	—	—	—	—	—
17:0 2-hydroxy	2.45	—	—	—	—	—	—
17:0 anteiso	14.52	—	—	—	—	—	—
2-hydroxy	—	—	—	—	—	—	—

Table 3. Menaquinone content in *Stackebrandtia* and *Glycomyces* species

Values are percentages of total menaquinones. Abbreviations are exemplified by MK-9(H₄), a menaquinone which has two of the nine isoprene units hydrogenated. Species: 1, *Stackebrandtia nassauensis* NRRL B-16338^T; 2, *Glycomyces algeriensis* NRRL B-16327^T; 3, *G. arizonensis* NRRL B-16153^T; 4, *G. harbinensis* NRRL 15337^T (determined by mass spectrometry; data from Labeda *et al.*, 1985); 5, *G. lechevalierae* NRRL B-16149^T; 6, *G. rutgersensis* NRRL B-16106^T (determined by mass spectrometry; data from Evtushenko *et al.*, 1991; Labeda *et al.*, 1985); 7, *G. tenuis* NRRL B-16895^T (determined by mass spectrometry; data from Evtushenko *et al.*, 1991). TR, Trace amount.

Menaquinone	1	2	3	4	5	6	7
MK-9(H ₆)	—	—	—	—	—	—	±
MK-10	—	23	—	—	17	—	—
MK-10(H ₂)	—	—	18	+	21	+	—
MK-10(H ₄)	30	—	52	—	11	—	—
MK-10(H ₆)	16	—	TR	+	—	±	+
MK-11	—	65	—	—	24	—	—
MK-11(H ₂)	—	—	9	—	19	—	—
MK-11(H ₄)	31	—	22	—	8	—	—
MK11(H ₆)	23	—	—	—	—	—	+
MK-12	—	12	—	—	TR	—	—

Fig. 2. Scanning electron micrograph of 21-day growth of *Stackebrandtia nassauensis* NRRL B-16338^T on ISP-2 agar. Bar, 10 μ m.

suborder *Glycomycineae* Rainey *et al.* 1997, but it is premature to base this proposal on a genus containing one species. The description of the family *Glycomycetaceae* is therefore emended to permit inclusion of the genus *Stackebrandtia*.

Description of *Stackebrandtia* gen. nov.

Stackebrandtia (Stack.e.brandt'i.a. N.L. fem. n. *Stackebrandtia* named for Erko Stackebrandt, a German microbiologist who has contributed significantly to the molecular systematics of prokaryotes, including actinobacteria).

Aerobic. Gram-positive, non-motile actinomycetes. Branched substrate mycelium (approx. 0.5 μ m in diameter) and, on some media, aerial mycelia are produced. Mycolic acids are absent. Catalase-positive. Contains *meso*-diaminopimelic acid as the diamino acid. Cell walls contain *N*-glycolylmuramic acid. The whole-cell sugar pattern consists of ribose and inositol, with traces of arabinose and mannose. The phospholipid pattern consists of phosphatidylglycerol, diphosphatidylglycerol and two additional unknown phospholipids. The predominant menaquinones are MK-10(H₄), MK-10(H₆), MK-11(H₄) and MK-11(H₆). Fatty-acid profile is rich in branched-chain and saturated components including 10-methyl-branched heptadecanoic acid and iso-branched 2-hydroxy fatty acids. Phylogenetically, the nearest neighbour is the genus *Glycomyces*. Type species is *Stackebrandtia nassauensis*.

Description of *Stackebrandtia nassauensis* sp. nov.

Stackebrandtia nassauensis (nas.sau.en'sis. N.L. fem. adj. *nassauensis* from Nassau, named after the place of origin of the type strain, Nassau, Providence, Bahamas).

Displays the following properties in addition to those given in the genus description. Pale yellow to pale tan vegetative mycelium; white to yellowish-white aerial hyphae produced on most media, especially ISP-2. Degrades or hydrolyses

allantoin, casein, aesculin, gelatin, hypoxanthine, starch and tyrosine. No degradation of adenine or xanthine. Produces phosphatase. Weakly reduces nitrates. Weakly assimilates acetate and malate; does not assimilate benzoate, citrate, lactate, mucate, oxalate, propionate, succinate or tartrate. Acid is produced from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, lactose, maltose, mannose, melibiose, methyl α -D-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose and xylose; no acid produced from adonitol, dulcitol, erythritol, inositol, mannitol, melezitose or methyl β -xyloside. Temperature range for growth is 15–37 °C. Grows in the presence of 4–9% NaCl. G+C content of the DNA is 72.4 mol% (HPLC).

The type strain, NRRL B-16338^T (LLR-40K-21^T = DSM 44728^T), was isolated by Mary Lechevalier from a soil sample from Nassau, Bahamas.

Emended description of the family *Glycomycetaceae* Rainey *et al.* 1997

Glycomycetaceae (Gly.co.my.ce.ta'ceae. N.L. masc. n. *Glycomyces* type genus of the family; -taceae ending to denote a family; N.L. fem. pl. n. *Glycomycetaceae* the *Glycomyces* family).

Aerobic. Gram-positive, non-acid-fast, non-motile actinomycetes comprising the genera *Glycomyces* and *Stackebrandtia*. Branched substrate mycelium (approx. 0.35–0.5 μ m in diameter) and, on some media, aerial mycelia are produced. Mycolic acids are absent. Catalase-positive. Contains *meso*-diaminopimelic acid as the diamino acid. Cell walls contain *N*-glycolylmuramic acid. Whole-cell sugar pattern contains ribose as a diagnostic sugar along with other genus-specific sugars. Phospholipid content includes phosphatidylglycerol and diphosphatidylglycerol in addition to other genus-specific phospholipids. The 16S rRNA gene sequence pattern of 16S rRNA signature nucleotides contains 70–98 (A–U), 415 (C), 449 (C), 534 (G), 681–709 (A–U), 825–875 (G–C), 999–1041 (C–G), 1059–1198 (C–G), 1064–1192 (G–G), 1117–1183 (A–U) and 1309–1328 (C–G). The type genus is *Glycomyces*.

Acknowledgements

The able technical assistance of E. N. Hoekstra for physiological characterization, DNA isolation and purification and 16S rRNA gene sequencing is gratefully acknowledged. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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