Glycomyces, a New Genus of the *Actinomycetales*

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We describe two species of the new genus *Glycomyces*, *Glycomyces harbinensis* sp. nov. and *Glycomyces rutgersensis* sp. nov. Members of this genus are aerobic, produce nonfragmenting vegetative hyphae, and form chains of conidia on aerial sporophores. The cell walls are type II (*meso*-diaminopimelic acid and glycine are present), and the whole-cell sugar patterns are type D (xylose and arabinose are present). The phospholipid pattern of both species is type P-I (no nitrogenous phospholipids). The guanine-plus-cytosine content of the deoxyribonucleic acid ranges from 71 to 73 mol%. The type strain of type species *G. harbinensis* is strain NRRL 15337 (= LL-D05139), and the type strain of *G. rutgersensis* is strain NRRL B-16106 (= LL-I-20).

During the course of isolation of actinomycete strains from soil for an antibiotic screening program, a novel isolate was obtained from a soil sample from Harbin, People's Republic of China. This culture produced the antibiotic azaserine and a novel antibiotic which is the subject of a United States patent application. The isolate formed short chains of conidia borne on aerial hyphae without fragmentation of the substrate mycelium, morphology similar to that found in the genera *Actinomadura* and *Micropolyspora*. The strain had type II cell wall chemistry (*meso*-diaminopimelic acid and glycine present) rather than type III or IV (*meso*-diaminopimelic acid present, but no glycine) (cell wall characteristic of *Actinomadura* and *Micropolyspora*, respectively). Moreover, the whole-cell sugar pattern of this strain consisted of arabinose and xylose, which is typical of members of the family *Actinoplanaceae* (which produce spores in sporangia) and members of the family *Micromonosporaceae* (which produce single spores on single sporophores). Neither of these families contain any genus which could accommodate a strain exhibiting the morphological properties described above. Another strain, which exhibited similar chemotaxonomic and morphological characteristics, was isolated from a sample of greenhouse soil from Trenton, N.J. We propose to include these strains in the new genus *Glycomyces*; strain NRRL 15337 is designated the type strain of the type species, *Glycomyces harbinensis*, and strain NRRL B-16106 is designated the type strain of the additional new species, *Glycomyces rutgersensis*.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains NRRL 15337^T (T = type strain) and NRRL B-16106^T were maintained as slant cultures on ATCC 172 medium (1), as frozen seeds in 10% glycerol, or as lyophilized preparations. The other media used in this study included the media recommended for characterization of *Streptomyces* species (21, 25), including Bennett agar, calcium malate agar, Czapek agar, Hickey-Tresner agar, inorganic salts-starch agar, oatmeal agar, and yeast extract-malt extract agar. Gauze no. 1 mineral salts agar (6) and ATCC 172 medium (1) were also included in the study.

Physiological properties. The media and tests used to evaluate physiological properties were those described by

Gordon et al. (8). Esculin hydrolysis was evaluated by the method of Williams et al. (27), and Tween 80 hydrolysis was evaluated by the method of Sierra (26). Phosphatase activity was evaluated on ATCC 172 medium amended with 0.1% phenolphthalein diphosphate, as described by Kurup and Schmitt (11).

Cell chemistry. Whole-cell chemistry and cell wall chemistry were analyzed by the methods of Becker et al. (2, 3) and Lechevalier et al. (13, 14); lipid composition was determined by the methods of Lechevalier et al. (15, 16). Menaquinones were extracted, separated, and purified by the procedures of Collins et al. (4, 5); they were identified from the masses of their molecular ions, which were determined with a Finnigan quadrupole MS/MS mass spectrometer.

DNA isolation and characterization and DNA-DNA hybridization study. Deoxyribonucleic acid (DNA) was extracted by the method of Marmur (17) and was purified by dihydroxyapatite chromatography. The guanine-plus-cytosine (G+C) content of the purified DNA was calculated from its buoyant density, which was determined by CsCl density gradient centrifugation in a Beckman model E analytical ultracentrifuge (22), and from its thermal denaturation midpoint in 0.1× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) by the method of Marmur and Doty (18). The reference DNA used for buoyant density determinations was from *Tilletia caries* NRRL Y-12807; this DNA had a buoyant density in CsCl of 1.71567 g/cm³ (C. P. Kurtzman, unpublished data). The extent of DNA homology between strains was determined spectrophotometrically by the method of Seidler et al. (23, 24), as described by Kurtzman et al. (10).

Electron microscopy. Samples for scanning electron microscopy were prepared from agar blocks containing colonial growth, which were fixed overnight with osmium tetroxide vapors. The blocks were trimmed, dehydrated through a graded acetone series, and then critical-point dried from liquid carbon dioxide. The dried colonies were then mounted on aluminum stubs with colloidal silver paste and sputter-coated with approximately 12.5 nm of gold-palladium. The samples were observed at 20 keV by using an ISI model SS-130 scanning electron microscope fitted with a lanthanum hexaboride emitter.

RESULTS

Micromorphology. The vegetative hyphae of neither strain fragmented during growth. The aerial hyphae of both strain NRRL 15337^T and strain NRRL B-16106^T fragmented into

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FIG. 1. Scanning electron micrograph of 14-day growth of *G. harbinensis* NRRL 15337^T on Czapek agar. Note the fragmentation of the aerial hyphae into square-ended conidia. Bar = 2 μ m.

short chains of conidia. This was clearly observed in scanning electron micrographs of 14-day growth of both strains (Fig. 1 and 2). The vegetative mycelia were branched and approximately 0.5 μ m in diameter; the spores were ovoid and 0.45 to 0.55 by 1.0 to 1.1 μ m and had smooth surfaces.

Gross colonial morphology. The vegetative mycelium of *G. harbinensis* on most media was white to yellowish white (Table 1). When produced, the aerial mycelium was white, and a slight yellowish soluble pigment was produced on ATCC 172 agar medium. Except for more prolific production of aerial mycelium and the production of a yellowish to brown soluble pigment on several media, *G. rutgersensis* closely resembled the type species in appearance. Both strains produced a fetid odor on yeast extract-malt extract agar.

Cell chemistry. The cell walls of strains NRRL 15337^T and NRRL B-16106^T contained *meso*-diaminopimelic acid and glycine, as well as glucosamine, muramic acid, glutamic acid, and alanine, which is typical of type II cell walls (13, 14). The whole-cell sugar pattern for these strains consisted of xylose and arabinose as characteristic sugars (type D [13, 14]). The phospholipid pattern for both strains consisted of phosphatidylinositol, diphosphatidylglycerol, and numerous acylated phosphatidylinositol mannosides, which is characteristic of type PI phospholipids (15, 16). The menaquinones found in extracts of these strains were MK-10(H2) and

MK-10(H6). Mycolates were absent. The G+C contents of these strains ranged from 71 to 73 mol%.

Physiology. The physiological differences between *G. harbinensis* and *G. rutgersensis* are summarized in Table 2. Both of the strains examined hydrolyzed casein, esculin, starch, adenine, and hypoxanthine. Neither strain hydrolyzed tyrosine, xanthine, or urea. Both strains produced phosphatase and grew in the presence of 5% NaCl, but neither strain grew in lysozyme broth. Both strains utilized acetate, DL-malate, and propionate; neither utilized benzoate, mucate, oxalate, or tartrate. Both strains produced acid from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, maltose, mannose, alpha-methylglucoside, beta-methylxyloside, raffinose, rhamnose, salicin, sorbitol, trehalose, and xylose; neither produced acid from dulcitol, erythritol, inositol, or sucrose.

DNA-DNA homology. Reassociation studies showed that the percentage of homologous DNA in these strains ranged from 21.5 to 30.2% (based on four separate determinations).

DISCUSSION

Several genera of the *Actinomycetales* are morphologically similar to the strains described above in the production of chains of conidia on their aerial hyphae. Nevertheless, strains NRRL 15337^T and NRRL B-16106^T are distinguished by their unique morphological and chemotaxonomic profiles,

FIG. 2. Scanning electron micrograph of 14-day growth of *G. rutgersensis* NRRL B-16106^T on yeast extract-malt extract agar. Aerial mycelium has fragmented into square-ended conidia. Bar = 1 μ m.

which support the conclusion that they do not belong to any previously described genus (7, 12, 19, 20) (Table 3). Although species belonging to the genera *Actinomadura* and *Nocardioidea* possess a type PI phospholipid pattern (no nitrogenous phospholipids are present), they do not have type II cell wall chemistry (i.e., *meso*-diaminopimelic acid is present but glycine is not), a characteristic that distinguishes these two genera from all other conidium-producing genera except "*Catenuloplanes*" (W. D. Celmer et al., U.S. patent

TABLE 2. Comparison of the physiological reactions of *G. harbinensis* NRRL 15337^T and *G. rutgersensis* NRRL B-16106^T

Characteristic	<i>G. harbinensis</i> NRRL 15337 ^T	<i>G. rutgersensis</i> NRRL B-16106 ^T
Dissimilation of gelatin	— ^a	±
Production of nitrate reductase	±	+
Utilization of:		
Citrate	—	+
Lactate	+	—
Succinate	—	+
Tartrate	—	—
Acid produced from:		
Adonitol	—	+
Lactose	±	+
Mannitol	+	—
Melezitose	±	—
Melibiose	—	+
Growth at:		
37°C	+	+
42°C	—	+
45°C	—	—

^a +, Positive; ±, weak activity; —, negative.

4,287,182, September 1981). They differ from "*Catenuloplanes*" in that members of the latter taxon have a type PIII phospholipid pattern (phosphatidylcholine is present) and also produce motile spores. Motile spores were not observed in either of the strains described in this paper. Consequently, we propose a new genus, *Glycomyces*, to accommodate these strains, with *Glycomyces harbinensis* NRRL 15337 as the type strain of the type species. On the basis of phenotypic characteristics and, more importantly, low levels of DNA relatedness, we propose that strain NRRL B-16106^T is a strain of another new species, *Glycomyces rutgersensis*, and should be designated its type strain. Formal descriptions of the genus and two species are given below.

***Glycomyces* gen. nov.** *Glycomyces* (Gly co. my. ces. Gr. adj. *glykus* sweet; *myke* fungus; Gr. n. *Glycomyces* sweet [glycolipid-containing] fungus) vegetative mycelia are branching (diameter, approximately 0.35 to 0.40 μ m); aerial mycelium is produced on certain growth media. No fragmentation of the vegetative hyphae occurs, but chains of square-ended conidia are produced on the aerial hyphae. Gram positive. Lysozyme sensitive. Catalase positive and aerobic.

TABLE 1. Cultural characteristics of *G. harbinensis* NRRL 15337^T

Medium	Amt of growth	Characteristics of vegetative and aerial mycelia ^a	Soluble pigments
Bennett agar	Moderate	Raised, waxy, yellowish white (92) vegetative growth; no aerial mycelium; fetid odor	None
Calcium malate agar	Good to moderate	White vegetative mycelium with sparse white aerial hyphae	None
Czapek sucrose agar	Good to moderate	White to pale yellow (89) vegetative mycelium with copious white aerial hyphae and spores	None
Gauze no. 1 agar	Moderate	Flat, dull, colorless vegetative mycelium; no aerial hyphae	None
Hickey-Tresner agar	Moderate	Plicate, waxy, yellowish white (92) vegetative growth; no aerial hyphae; fetid odor	None
Inorganic salts-starch agar	Poor to moderate	Flat, colorless to white vegetative mycelium with sparse white aerial hyphae around edges of colonies	None
ATCC 172 agar medium	Good	Raised, ridged, yellowish white (92) vegetative mycelium; trace of white aerial hyphae	Yellow
Oatmeal agar	Moderate	Flat, colorless to white vegetative mycelium; no aerial hyphae	None
Yeast extract-malt extract agar	Good	Plicate, raised, yellowish white (92) to pale yellow (89) vegetative mycelium; no aerial hyphae	None

^a The numbers in parentheses are color designations taken from reference 9.

TABLE 3. Comparison of *Glycomyces* with other related genera of the *Actinomycetales* which form chains of spores on their aerial hyphae

Genus	Fragmentation of vegetative mycelia	Motile spores	Cell wall type	Whole-cell sugar(s)	Phospholipid group	Principal menaquinone(s)	DNA G+C content (mol%)
<i>Glycomyces</i>	—	—	II	Xylose, arabinose	PI	MK-10(H2), MK-10(H4)	71-73
<i>Actinomadura</i>	—	—	III	Madurose	PI	MK-9(H4), MK-9(H6)	65.5-69.7
" <i>Catenuloplanes</i> "	—	+	II	Xylose, arabinose	PIII	NA ^a	NA
<i>Nocardioidea</i>	+	—	I	None	PI	NA	65.5
<i>Nocardioopsis</i>	+	—	III	None	PII	MK-10(H4), MK-10(H6)	64-69
<i>Saccharothrix</i>	+	—	III	Rhamnose, galactose	PII	MK-9(H4)	73

^a NA, Data not available.

Type II cell wall composition (*meso*-diaminopimelic acid and glycine) and whole-cell sugar pattern consisting of xylose and arabinose (type D). Type PI phospholipid pattern, with significant amounts of phosphatidylinositol mannosides. The principal menaquinones present are MK-10(H2) and MK-10(H6). The type species is *Glycomyces harbinensis*.

Glycomyces harbinensis sp. nov. *Glycomyces harbinensis* (har. bin. en sis. L. adj. *harbinensis* referring to Harbin, People's Republic of China, the source of the soil sample from which the organism was first isolated) substrate mycelium is pale yellow to yellowish white; when produced (e.g., in Czapek sucrose agar), the aerial mycelium is white. Soluble pigments are not produced on most media; a yellow soluble pigment is produced on ATCC 172 medium. Casein, esculin, starch, adenine, and hypoxanthine are hydrolyzed; gelatin, tyrosine, xanthine, and urea are not hydrolyzed. Phosphatase is produced; nitrate reductase activity is weak. Growth in the presence of 5% NaCl. Assimilation of acetate, citrate, DL-malate, propionate, and succinate; no assimilation of benzoate, lactate, mucate, oxalate, or tartrate. Acid is produced from adonitol, arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, lactose, maltose, mannose, alpha-methyl-D-glucoside, beta-methylxyloside, raffinose, rhamnose, salicin, sorbitol, trehalose, and xylose; no acid production from dulcitol, erythritol, inositol, mannitol, melezitose, melibiose, or sucrose. Temperature range for growth, 20 to 42°C. G+C content of DNA, 71 mol% (thermal denaturation midpoint method). Isolated from soil. The type strain of *G. harbinensis* is strain NRRL 15337 (= LL-D05139).

Glycomyces rutgersensis sp. nov. *Glycomyces rutgersensis* (rut. gers. en sis. L. adj. *rutgersensis* referring to Rutgers University, where the organism was first isolated) substrate mycelium ranges from yellowish white to tan, depending on the medium; white aerial hyphae are produced on several media (e.g., ATCC 172 medium). Yellowish to brown soluble pigments are produced on several media. Casein, esculin, gelatin, starch, adenine, and hypoxanthine are hydrolyzed; tyrosine, xanthine, and urea are not hydrolyzed. Phosphatase and nitrate reductase activity produced. Growth in the presence of 5% NaCl. Assimilation of acetate, lactate, DL-malate, and propionate; no assimilation of benzoate, citrate, mucate, oxalate, succinate, or tartrate. Acid produced from arabinose, cellobiose, dextrin, fructose, galactose, glucose, glycerol, maltose, mannitol, mannose, alpha-methyl-D-glucoside, beta-methylxyloside, raffinose, rhamnose, salicin, sorbitol, trehalose, and xylose; weak to variable acid production from lactose and melezitose; no acid production from adonitol, dulcitol, erythritol, inositol, melibiose, or sucrose. Temperature range for growth, 20 to

37°C. G+C content of DNA, 73 mol% (thermal denaturation midpoint method) or 70 mol% (buoyant density method). Isolated from soil. The type strain of *G. rutgersensis* is strain NRRL B-16106 (= LL-120).

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