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Novel Neogala-Series Glycosphingolipids with a Terminal Glucose Residue from the Fungus *Mariannaea elegans*

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Glycosphingolipids (GSLs) are essential membrane components of eukaryotic cells. Recently, a new type of fungal neogala-series GSL was identified in aureobasidin A-resistant fungi. In this study, we analyzed GSLs from four pathogenic fungal strains belonging to the order Hypocreales, and found that *Mariannaea elegans* contained both acidic GSLs and neutral GSLs with mono- and di-saccharides. The structures of the neutral GSLs of *M. elegans* were determined by compositional sugar, fatty acid, and sphingoid analyses by GC/MS, MALDI time-of-flight/MS, and ^{1}H NMR. The ceramide moiety of Glcβ1-Cer consisted mainly of the 2-hydroxylated $C_{18:0}$-fatty acid 9-methyl-octadeca-4-sphinganine or 9-methyl-octadeca-4,8-sphingadienine. In contrast, the ceramides of Galβ1-6Galβ1-Cer and Glc1-6Galβ1-Cer consisted mainly of saturated 2-hydroxylated $C_{24:0}$-fatty acids and $C_{18:0}$-phytosphingosine. To our knowledge, Glc1-6Galβ1-Cer is a novel GSL in fungi, and *M. elegans* is the first example of an aureobasidin A-sensitive fungus that possesses fungal neogala series GSLs.

Key words: aureobasidin A; fungal neogala-series glycosphingolipids; glycosylinositolphosphoceramides; Hypocreales

Glycosphingolipids consist of a hydrophilic carbohydrate chain of variable length and structure linked to a hydrophobic ceramide (*N*-acylsphingosine) moiety.[1,2] Two distinct types of GSLs have been known for many years to occur ubiquitously in fungi.[3] One class of fungal GSLs is the neutral monoglycosylceramides (ceramide monohexosides), including glucosylceramide and galactosylceramide, which contain a sphingoid base in the ceramide moiety.[4] The other is the acidic glycosylinositolphosphoceramides (GIPCs), which contain a phytosphingoid base constituting the core structure of inositolphosphoceramide (IPC) with the addition of mannose, galactosamine, galactose, and/or xylose.[5–7]

Recently, a new type of fungal neogala-series GSL (FNG-GSL) was identified in several fungal species, including *Mucor hiemalis*, *Rhizopus microsporus*, *Rhizomucor pusillus*, *Absidia corymbifera*,[8] and *Hirsutella rhossiliensis*.[9] FNG-GSLs contain a characteristic fungal neogala-series core structure, Galβ1-6Galβ1-Cer, with a phytosphingoid base, as seen in Manα1-3Galβ1-6Galβ1-6Galβ1-Cer, Glcα1-2Galβ1-6Galβ1-6Galβ1-Cer, and Manα1-3Galβ1-6(Glcα1-4)Galβ1-6Galβ1-Cer of *H. rhossiliensis*, and Galα1-6Galα1-6Galα1-6Galβ1-6Galβ1-Cer of *M. hiemalis*. The carbohydrate chains of FNG-GSLs vary in length, combination, sequence, and branch structure, in contrast to most fungal GSLs, which are glycosylated with only a single sugar. FNG-GSLs are also different from the neogala-series GSLs found in the earthworm *Pheretima* sp., especially in the type of terminal glycosidic bonds (α1-3-linked Man and α1-2-linked Glc in the fungi, and α1-4-linked Man and Glc in the earthworm) in their carbohydrate chains.[10,11]

The fungi thus far found to have FNG-GSLs without exception exhibit resistance to aureobasidin A (AbA). This is a well-known antifungal agent that exhibits strong activity against many fungi by inhibiting IPC synthase,[12–14] which catalyzes the transfer of inositol phosphate from phosphatidylinositol to phytoceramide to form IPC, an essential step in the biosynthesis of fungal GIPCs. Recent studies have revealed that AbA-resistant fungi do not have the GIPC synthetic pathway. Instead they contain FNG-GSLs.[8,9] The evidence suggests that AbA-resistant fungi substitute FNG-GSLs for GIPCs in various cellular roles, but the distribution of FNG-GSLs within the fungal kingdom and the correlation between FNG-GSLs and AbA-resistance are still unclear.

Structural analyses of the neutral GSLs revealed that the fungus has several FNG-GSLs, including one with a completely novel structure, Glc1-6Galβ1-Cer. In addition, we found that the growth of *M. elegans* was strongly inhibited by AbA, suggesting the presence of GIPCs in the fungus. Thus *M. elegans* is the first example of an AbA-sensitive fungus that possesses FNG-GSLs.

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Abbreviations: AbA, aureobasidin A; Cer, ceramide; FNG-GSL, fungal neogala-series GSL; GC/MS, gas chromatography/mass spectrometry; GSL, glycosphingolipid; GIPC, glycosylinositolphosphoceramide; IPC, inositolphosphoceramide; MALDI-TOF/MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; NGL, neutral glycolipid

Materials and Methods

Culture of fungal strains. Ascomycota strains *M. elegans* JCM12789 and *Aspergillus oryzae* RIB40 were obtained from the Japan Collection of Microorganisms (Wako, Japan) and the National Research Institute for Brewing (Higashi-hiroshima, Japan). The fungal strains were cultivated in YPG medium (0.5% yeast extract, 0.5% peptone, 1% glucose, and 0.5% NaCl) at 30 °C for 6 d in a 2-L shaking flask containing 500 mL of medium at 200 rpm. For the growth inhibition assay with AbA, fungal strains were grown on YPG agar plates containing 10 μg/mL of AbA (Takara Bio, Ohtsu, Japan) for 2 d at 30 °C.[8,9]

Extraction and purification of GSLs. The cultivated mycelia were harvested and freeze dried. The dried powder (56 g) was extracted twice with chloroform–methanol–water (1:2:0.8, v/v) at 37 °C for 20 h. The extract was dried and subjected to mild alkaline hydrolysis with 0.5 M KOH in methanol–water (95:5, v/v) at 37 °C for 12 h. The hydrolysate was acidified to pH 1.0 with concentrated HCl, and then dialyzed against tap water for 2 d. Glycolipids in the dialysate were precipitated with acetone, dissolved in choloroform–methanol (2:1, v/v), and applied to a QAE-Sephadex A-25 column (GE Healthcare, Tokyo). Elution was carried out with 5 volumes of chloroform–methanol–water (30:60:8, v/v) to obtain a neutral GSL fraction. Polar compounds were eluted with 0.005–0.45 M ammonium acetate in methanol. The neutral GSLs were acetylated with acetic anhydride–pyridine (3:2, v/v) at 20 °C for 18 h, purified on a Florisil column (Nacalai Tesque, Kyoto, Japan), deacetylated with 0.5 M KOH in methanol at 37 °C for 6 h, and dialyzed against tap water for 2 d. The neutral GSLs were applied to a column of porous silica gel (Iatrobeads 6RS-8060, Mitsubishi Chemical Medience, Tokyo), and eluted with chloroform–methanol–water (70:30:3, v/v).

Analysis of fatty acid, sphingoid, and sugar compositions of the neutral GSLs. Purified GSLs (50–100 μg) were methanolyzed with 200 μL of freshly prepared 1 M anhydrous methanolic HCl in a thick glass test tube at 100 °C for 3 h. After methanolysis, the fatty acid methylesters were extracted 5 times with 200 μL of *n*-hexane and analyzed by capillary gas chromatography/mass spectrometry (GC/MS). The remaining methanolic phase was evaporated to dryness for deacidification under a nitrogen stream. The residue containing methylglycosides was trimethylsilylated and subjected to gas chromatography (GC) analysis.

Methylation analysis to determine sugar linkages. To identify the sugar linkages of the oligosaccharides in the neutral GSLs, 100 μg of the purified GSL was partially methylated with NaOH and CH_3I in DMSO.[15] The permethylated GSL was hydrolyzed with 300 μL of HCl–water–acetic acid (0.5:1.5:8, v/v) at 80 °C for 18 h, and then reduced with $NaBH_4$ and acetylated with acetic anhydride–pyridine (1:1, v/v) at 100 °C for 15 min. The partially methylated alditol acetates were analyzed by GC and GC/MS.

Thin-layer chromatography. Silica gel 60 TLC plates (Merck, Darmstadt, Germany) were developed to a distance of 5 cm with chloroform–methanol–water (60:35:8, v/v). Detection was performed with the orcinol–H_2SO_4 reagent for sugar, the Dittmer–Lester reagent for phosphorus,[16] and the ninhydrin reagent for free amino groups.

GC and GC/MS. A Shimadzu GC-18A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a capillary column (Shimadzu HiCap-CBP 5, 0.22 mm × 25 m) was used to analyze sugar linkages and the compositions of sugars and fatty acids. The following temperature programs were used: 140 °C to 230 °C (2 °C/min) for sugar trimethylsilyl derivatives, 170 °C to 230 °C (2 °C/min) for fatty acid methyl esters, 140 °C to 230 °C (4 °C/min) for partially methylated alditol acetate derivatives, and 210 °C to 230 °C (2 °C/min) for sphingoid trimethylsilyl derivatives. Electron impact mass spectra were obtained with a Shimadzu GCMS-QP 5050 GC/MS system (Shimadzu) under the following conditions: interface temperature 250 °C, injection port temperature 240 °C, helium gas pressure 100 kPa, ionizing voltage 70 eV, and ionizing current 60 μA. The oven temperatures for GC/MS analysis were 80 °C (2 min) to 170 °C (20 °C/min) to 240 °C (4 °C/min) for sugar trimethylsilyl derivatives, 80 °C (2 min) to 170 °C (20 °C/min) to 240 °C (4 °C/min) for fatty acid methyl esters, 80 °C (2 min) to 160 °C (20 °C/min) to 240 °C (4 °C/min) for partially methylated alditol acetate derivatives, and 80 °C (2 min) to 210 °C (20 °C/min) to 230 °C (4 °C/min) for sphingoid trimethylsilyl derivatives.

MALDI time-of-flight (TOF)/MS. MALDI-TOF/MS analyses of neutral GSLs were performed with an Applied Biosystems/Voyager-DE STR Biospectrometer with a nitrogen laser (337 nm) at an acceleration voltage of 20 kV, operating in the reflector and post-source decay positive-ion mode. The matrix used was α-cyano-4-hydroxycinnanic acid (proteomics grade, Wako Pure Chemical Industries, Osaka, Japan). External mass calibration was done *via* the $[M + Na]^+$ ions of angiotensin 1 (1,296.69 mass units, proteomics grade; Wako) and bradykinin fragments 1–5 (573.31 mass units; Sigma Chemical, St. Louis, MO).

^1H NMR spectroscopy. NMR spectra of neutral GSLs were obtained with a JEOL JNM-ECS 400 MHz ^1H NMR spectrometer at an operating temperature of 60 °C (JEOL, Tokyo). The purified neutral GSL was dissolved in 0.6 mL of dimethylsulfoxide-d_6 containing 2% D_2O, and the chemical shift was referenced to the solvent signals ($d_H = 2.49$ ppm) in DMSO-d_6 as internal standard.

Results and Discussion

GSLs of various fungi

Several fungal species belonging to the phylum Zygomycota have been found to be resistant to AbA, and these AbA-resistant fungi contained FNG-GSLs, instead of GIPCs, in their membranes.[8] Further investigation revealed that *H. rhossiliensis*, within the phylum Ascomycota, also exhibits resistance to AbA and contains five different FNG-GSLs, including three novel GSLs.[9] Based on the infectious and pathogenic capacity of *H. rhossiliensis* and the unusual structures of its FNG-GSLs, we propose that the unique FNG-GSLs in *H. rhossiliensis* have a role in protecting the pathogen against the immune systems of host cells. Alternatively, FNG-GSLs themselves may act as specific toxins for insects and nematodes. Hence we speculated that other infectious fungi belonging to Hypocreales also contain FNG-GSLs or other unusual GSLs.

We examined the four Hypocreales strains, *Simplicillium lamellicola*, *Pochonia suchlasporia*, *Nectria gracilipes*, and *M. elegans*, for the presence of FNG-GSLs in their membranes. GSLs were extracted from each fungi cultured in an optimum medium and analyzed by TLC by the chloroform–methanol–water system. The GSLs from *S. lamellicola* and *P. suchlasporia* gave clearly visible spots corresponding to monoglycosylceramide, which is ubiquitously present in fungi. In contrast, the GSLs from *M. elegans* and *N. gracilipes* provided different TLC patterns from those observed for *S. lamellicola* and *P. suchlasporia*. The two spots obtained by performing TLC with *N. gracilipes* GSLs were assigned to mono- and di-glycosylceramide on the basis of mobility. On the other hand, the GSLs of *M. elegans* showed at least nine spots in addition to a large spot corresponding to monoglycosylceramide (Fig. 1A). This suggests that the composition of GSLs of *M. elegans* is complex and different from those of other fungi previously reported.[4,17]

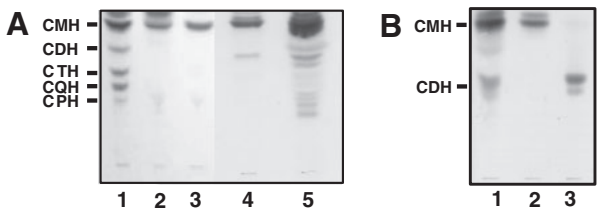

Fig. 1. TLC Analysis of GSLs Extracted from Hypocreales.
A, TLC of glycolipids from Hypocreales. Lanes 1–5 show glycolipids from *Rhizopus* sp. as control, *S. lamellicola*, *P. suchlasporia*, *N. gracilipes*, and *M. elegans* respectively. Orcinol–H_2SO_4 reagent was used for detection. B, TLC of neutral GSLs from *M. elegans*. Lane 1, neutral GSLs from *M. elegans*; lane 2, purified NGL1; lane 3, purified NGL2. TLC mobility is different between panel A and B due to slightly different conditions as to solvents. Orcinol–H_2SO_4 reagent was used for detection. CMH, CDH, CTH, CQH, and CPH are GSLs with mono-, di-, tri-, tetra-, and pentasaccharides.

Isolation of neutral GSLs from M. elegans

We extracted GSLs from the mycelia of *M. elegans* grown in YPG liquid medium. The GSLs were then separated on the basis of their polarities into neutral and acidic fractions by ion-exchange column chromatography. Each fraction was analyzed by TLC by a chloroform–methanol–water system. The GSLs in the neutral fraction consisted of two species, which were identified as monosaccharide- and disaccharide-containing GSLs on the basis of TLC mobility (Fig. 1B, lane 1). It is plausible to propose that the other spots in Fig. 1A, except for neutral spots, are acidic GSLs (data not shown). We tentatively designated these monosaccharide- and disaccharide-containing neutral GSL species NGL1 and NGL2 respectively. Each was further purified by silica-gel column chromatography. Both NGL1 and NGL2 were found to be pure on the basis of TLC analysis (Fig. 1B, lane 2 and lane 3). The amounts of NGL1 and NGL2 from 56 g of dried mycelia were 7.3 and 0.7 mg respectively.

Sugar, fatty acid, and sphingoid composition of the GSLs from M. elegans

To determine the chemical structures of NGL1 and NGL2, first we analyzed the composition of the sugar, fatty acid, and sphingoid components of these neutral GSLs. NGL1 and NGL2 were methanolyzed and then analyzed by GC and GC/MS. Glucose was the only sugar component found in NGL1 (Table 1). On the other hand, both glucose and galactose (in a ratio of 3:7) were detected in NGL2. NGL1 was also markedly different from NGL2 in the composition of its fatty acid and sphingoid components. The fatty acids of NGL1 consisted of 2-hydroxylated $C_{18:0}$-fatty acid (80.1%) and minor fatty acids including $C_{16:0}$-fatty acid (7.0%), $C_{18:0}$-fatty acid (4.9%), and unknown fatty acids (8.0%). In contrast, NGL2 contained long-chain fatty acids such as 2-hydroxylated $C_{24:0}$-fatty acids (64.3%) and 2-hydroxylated $C_{22:0}$-fatty acids (3.2%), in addition to the fatty acids seen in NGL1. The sphingoid moiety of NGL1 was determined to contain 9-methyl-octadeca-4,8-sphingadienine (47.3%) and 9-methyl-octadeca-4-sphinganine (23.4%) as the major species, whereas that of NGL2 contained only $C_{18:0}$-phytosphingosine. Thus NGL1 is distinct from NGL2 in that the former has sphingoid bases while the latter contains a phytosphingoid base. The ceramide composition of NGL1 is similar to the compositions of GSLs from fungal species in general, where these ceramides play important roles in fungal infections of plants.[18,19] On the other hand, the ceramide composition of NGL2 shows similarity to the FNG-GSLs of AbA-resistant fungal species[8,9,20] and the GIPCs of AbA-sensitive fungal species.[21–23]

Table 1. Compositions of the Ceramide Moieties of Purified GSLs from *M. elegans*

Component	Composition	NGL1	NGL2
Sugar (%)	Glc	100	30
	Gal	—[c]	70
Fatty acid[a] (%)	16:0	7.0	9.5
	18:0	4.9	6.2
	h18:0	80.1	11.0
	h22:0	—[c]	3.2
	h24:0	—[c]	64.3
	Unknown	8.0	5.8
Sphingoid[b] (%)	d19:1	23.4	—[c]
	d19:2	47.3	—[c]
	t18:0	—[c]	100
	Unknown	29.3	—[c]

[a]h, 2-hydroxy fatty acid
[b]d, dihydroxy sphingoid; t, trihydroxy sphingoid
[c]Not detected

MALDI-TOF/MS analysis

Next we analyzed the purified NGLs by positive-ion mode MALDI-TOF/MS. The presence of various fatty acid and sphingoid species generated mass spectra with several different pseudomolecular ions (Fig. 2A and B). We focused on the main peaks in the mass spectra. The mass spectrum of NGL1 showed two main peaks of $[M + Na]^+$ ions, at m/z 776.6 and 778.6 (Fig. 2A), which were consistent with the compositions of NGL1 as determined by the GC/MS analysis described above: one molecule of hexose, one molecule of 2-hydroxylated $C_{18:0}$-fatty acid, and one molecule of 9-methyl-octadeca-4,8-sphingadienine or 9-methyl-octadeca-4-sphinganine. Two ion peaks of $[M + Na]^+$, at m/z 1,002.7 and 1,030.8, observed in the NGL2 spectrum were consistent with the values calculated from their proposed structures: two hexose molecules, one molecule of 2-hydroxylated $C_{22:0}$-fatty acid or 2-hydroxylated $C_{24:0}$-fatty acid, and one molecule of $C_{18:0}$-phytosphingosine (Fig. 2B).

Methylation analysis of glycosidic linkages

To determine the glycosidic linkages in the sugar components of NGL1 and NGL2, we analyzed partially methylated alditol acetates derived from the NGLs by GC and GC/MS (Fig. 2C and D). With reference to the data obtained by the above-described methanolysis and MALDI-TOF/MS analyses, the peaks in GC analysis were assigned as follows: terminal Glc (1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucotitol, t-Glc) in NGL1 (Fig. 2C), a small amount of t-Glc, and large amounts of both terminal Gal (1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, t-Gal) and 6-substituted Gal (1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol, 6-Gal) in NGL2 (Fig. 2D). Therefore, NGL2 consists of two diglycosylceramide molecules with terminal sugars t-Gal and t-Glc. This indicates that the structure of NGL1 is

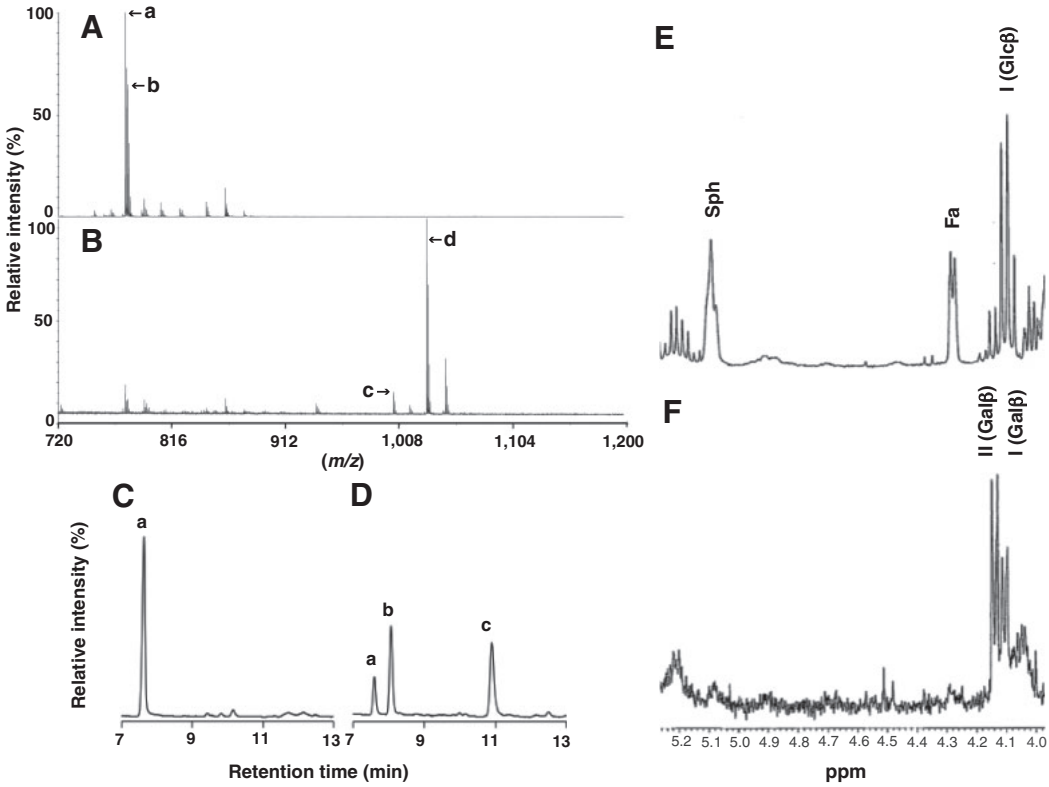

Fig. 2. Structure Analysis of GSLs from *M. elegans*.

A and B, MALDI-TOF/MS spectra of purified GSLs from *M. elegans*. Analyses were performed in positive-ion linear mode. Pseudomolecular ions are given in average masses. Panels A and B show spectra of NGL1 and NGL2 respectively. Multiple peaks (*a–d*) in the same panel contain different ceramide moieties. C and D, Gas chromatograms of partially methylated alditol acetates derived from purified GSLs for the determination of glycoside linkages. Panel C shows gas chromatograms of NGL1, and the peak of t-Glc is indicated by *a*. Panel D shows gas chromatograms of NGL2, and the peaks of t-Glc, t-Gal, and 6-Gal are indicated by *a*, *b*, and *c* respectively. E and F, ^{1}H-NMR spectra of purified GSLs from *M. elegans*. Anomeric proton regions of the spectra are shown (E, NGL1; F, NGL2). The resonances marked by I (Glcβ), I (Galβ), and II (Galβ) in panels E and F are due to the hexose moieties. The resonances marked by Sph and Fa in panel E are due to Δ8-unsaturation in sphingosine and saturated 2-hydroxy fatty acid.

glucosylceramide, and that the structures of NGL2 are Gal1-6Gal1-Cer and Glc1-6Gal1-Cer.

Anomeric configuration analysis of the sugar components of NGLs by ^{1}H NMR

To determine the anomeric configurations of the sugar residues, NGL1 and NGL2 were subjected to 400-MHz ^{1}H NMR spectroscopy. We also speculated as to the glycoside linkages in these compounds. In the anomeric regions of the spectra for the NGLs, anomeric proton resonances were observed at 4.11 ppm ($J_{1,2} = 7.79$ Hz) for β-Glc (Fig. 2E) and at 4.12 ppm and 4.15 ppm ($J_{1,2} = 6.87$ Hz and 6.87 Hz, respectively) for β-Gal (Fig. 2F). We are not able to assign a peak for the Glc residue of NGL2 in the NMR spectrum, most likely because it was buried under the peaks of larger amounts of β-Gal. Nevertheless, these results suggest that the Glc residue of NGL1 and the Gal residue of NGL2 are β-Glc and β-Gal respectively. Taken together, our results suggest that the structure of NGL1 is Glcβ1-Cer with a sphingoid base in the ceramide moiety, and that NGL2 includes Galβ1-6Galβ1-Cer and a small amount of Glc1-6Galβ1-Cer, both of which contain a phytosphingoid base.

Effect of AbA on the growth of M. elegans

Because we observed several spots that presumably corresponded to acidic GSLs in the TLC analysis of

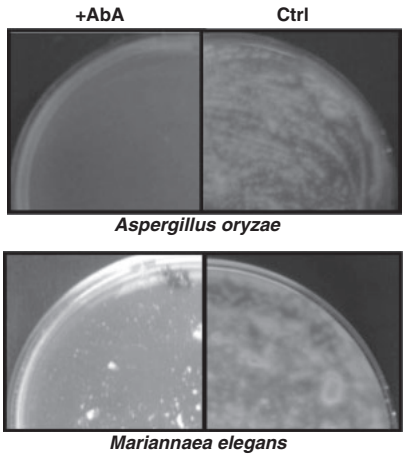

Fig. 3. Growth of Two Filamentous Fungi on the Medium Containing Aureobasidin A.

A. oryzae (upper panel) and *M. elegans* (lower panel) were grown on YPG medium plates for 2 d at 30 °C in the presence (+AbA) and the absence (Ctrl) of 10 μg/mL AbA.

GSLs from *M. elegans* (Fig. 1), we speculated that those acidic fractions contain GIPCs. A previous study found that the growth of GIPC-containing fungi, including *A. oryzae*, is impaired by AbA, which specifically inhibits the biosynthesis of GIPCs.[13,14] In contrast, the FNG-GSL-containing fungi thus far identified have no

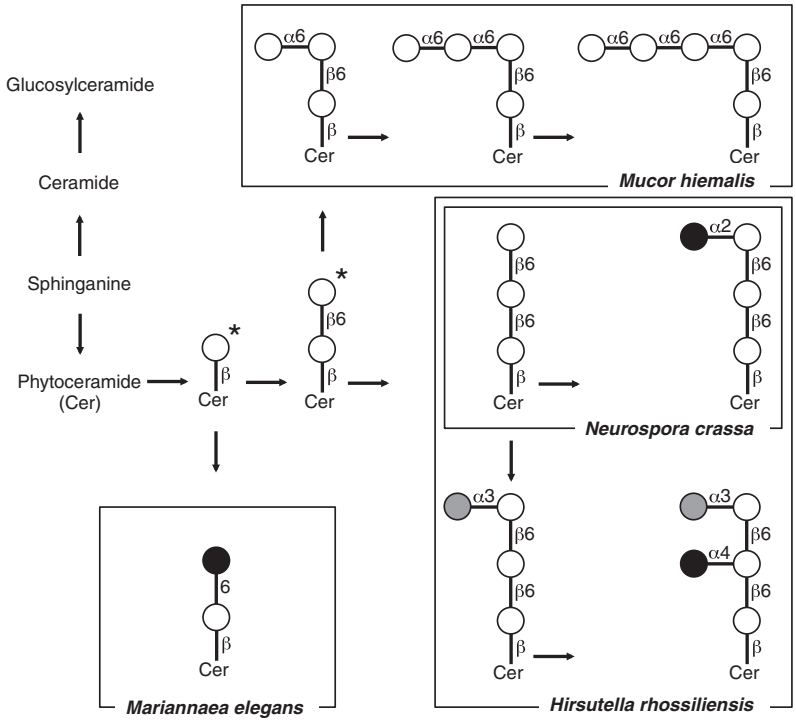

Fig. 4. Possible Biosynthetic Pathways of FNG-GSLs.

Structures of and proposed biosynthetic pathways for FNG-GSLs identified in *M. hiemalis*, *N. crassa*, *H. rhossiliensis*, and *M. elegans*. FNG-GSLs are depicted in a schematic drawing. Monosaccharides are represented by white circles (galactose), black circles (glucose), and gray circles (mannose). Cer represents a ceramide moiety with a phytosphingoid base (phytoceramide). The FNG-GSLs indicated by asterisks occurred in all four fungal strains, and represent neogala-series core structures. The linkages of the third Gal are α1-6 in *M. hiemalis* and β1-6 in *H. rhossiliensis* and *N. crassa*. In *M. hiemalis*, further elongation of α1-6-linked Gal residues occurred to form Galα1-6Galα1-6Galα1-6Galβ1-6Galβ1-Cer. In *N. crassa*, Galβ1-6Galβ1-6Galβ1-Cer is modified with a Glc residue. In *H. rhossiliensis*, Galβ1-6Galβ1-6Galβ1-Cer is modified with Man (and Glc). Galβ1-Cer is modified with Gal or Glc in *M. elegans*. Glucosylceramide is present in all four fungal strains.

GIPCs and exhibit AbA resistance.[8,9] To determine whether GIPCs are present in *M. elegans*, it was cultured on agar plates containing 10 μg/mL of AbA. We found that AbA significantly inhibited the growth of *M. elegans*, to an extent similar to *A. oryzae* (Fig. 3). This suggests that *M. elegans* has GIPCs as an essential membrane component for growth.

To date, FNG-GSLs have been identified only in AbA-resistant fungi.[8,9] Accordingly, we propose that such AbA-resistant fungi utilize FNG-GSLs instead of GIPCs invarious cellular events. Hence we propose that *M. elegans* is the first example of a fungus that contains both GIPCs and FNG-GSLs in the cell membrane. In contrast to the FNG-GSLs of AbA-resistant fungi, the FNG-GSLs of *M. elegans* do not substitute for GIPCs, suggesting that they are involved in an as-yet unidentified process in cell membranes, or can replace only some GIPC functions. Because one of the FNG-GSLs of *M. elegans*, Galβ1-6Galβ1-Cer, is equivalent to the core structure of the more complex FNG-GSLs of AbA-resistant fungi,[8,9] the extension of the carbohydrate chain (to more than 3 sugar units) in FNG-GSLs may be a prerequisite for using them as functional substitutes for GIPCs (Fig. 4).

Conclusion

In this study a series of neutral GSLs from *M. elegans* were analyzed. The major component of the neutral GSL fraction was determined to be Glcβ1-Cer (NGL1), and the minor components were Galβ1-6Galβ1-Cer and Glc1-6Galβ1-Cer (NGL2). Glc1-6Galβ1-Cer, a novel GSL in fungi, is completely different from the core structure Galβ1-6Galβ1-Cer of known FNG-GSLs (Fig. 4). In addition, we identified *M. elegans* as the first example of an AbA-sensitive fungus that possesses FNG-GSLs.

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