Structure Determination of Molecular Species of Glycosphingolipids From *Cladosporium herbarum* And Their Reactivities With a Monoclonal Antibody to Glucosylceramide (Glc-Cer)

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Fungi belonging to the genus *Cladosporium* are vastly distributed in nature. Some species are relevant as chromoblastomycosis agents or allergens (*C. herbarum*). Ceramide monohexosides are involved in morphological transition and fungal growth. Determination of structural and functional aspects of these glycoconjugates could contribute to the design of new agents capable of inhibiting fungal growth. *C. herbarum* mycelium was extracted with chloroform/methanol 2:1 and 1:2 (v:v).The crude lipid extract was partitioned according to Folch and coworkers and the lower phase was partially purified on silica gel column, eluted with chloroform, acetone and methanol. Acetone and methanol fractions containing the glycosphingolipids were further purified on silica gel column, which was eluted with chloroform/methanol with increasing concentrations of methanol (95:5, 9:1, 8:2. 1:1 v/v) and methanol. The fractions were analyzed by thin layer chromatography (TLC) and the spots were visualized with iodine and orcinol/H_2SO_4. The purified glycosphingolipids were analyzed by TLC and mass spectrometry and identified as molecules containing a glucose residue attached to 9-methyl-4,8-sphingadienine in amidic linkage to 2-hydroxyoctadecanoic or 2-hydroxyoctadecenoic acids. The purified molecules were recognized by monoclonal antibodies to glucosylceramide from *Aspergillus fumigatus*, suggesting that they are conserved in fungi. Finally, we observed by imunofluorescence that the presence of melanine-like pigments on conidia's surface interferes with recognition of CMH by monoclonal antibodies.

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