Comparative study of lipid composition of the mycelial cell wall from environmental and clinical isolates of *Histoplasma capsulatum*

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Objective

To perform a comparative study of lipid composition of mycelial cell wall from environmental and clinic isolates of *Histoplasma capsulatum* (*H. capsulatum*).

Methods

Fungal strains and growth conditions

H. capsulatum clinical isolate 1402065 was obtained by biopsy from a patient with histoplasmosis for the Grupo de Micología Médica and the *H. capsulatum* environmental isolate 316-1 was obtained from a soil amendment sample of the Grupo Interdisciplinario de Estudios Moleculares, Universidad de Antioquia. These isolates have been cultured their mycelial phase in Mycosel agar at 23°C.

Lipid extraction

The lipids were extracted from the mycelial phase of both *H. capsulatum* isolates. The mycelial fragments were mixed with hexane solvent for 6 months at 23°C. The growth of *H. capsulatum* was monitored monthly to check the fungal viability. Then the dead mycelial fragments were subjected to Soxhlet extraction, using hexane as the primary solvent. A second extraction was done with chloroform, followed by a third extraction with isopropanol and finally with methanol. The reflux time was 2 hours and the extracts were dried with nitrogen gas.

Derivatization and preparation of FAMES

The different extracts were treated with 4ml of 0.5N sodium hydroxide (NaOH) in methanol and placed in a water bath at 90 °C under a reflux system for 7 min. Thereafter, 20% boron trifluoride (BF₃) in methanol was added and maintained under a reflux system for 2 min. Then the samples were mixed with n-heptane and allowed to reflux for one additional min. The samples were removed from the water bath and allowed to stand for 5 min. Then of a saturated solution of NaCl was added to improve the separation of the organic phases. The organic phases were then collected into Eppendorf tubes with anhydrous sodium sulfate to dry the samples. The samples were centrifuged and a fraction of the sample was collected from the supernatant into auto-sampler glass-crimp cap vials (Agilent, Santa Clara, CA, USA) to undergo gas chromatography.

Gas chromatography

The internal standards mixture of FAMES in n-heptane (laureate, myristate, palmitate, stearate, oleate, linoleate, linolenate, arachidonate and docosahexaenoate) were runned before each assay to estimate the retention times, followed by the different extracts that were runned independently in the Agilent 6890N gas chromatograph with flame ionization detector (FID, Santa Clara, CA, USA). The chromatographic running was performed in a FAME column (FAME model JIWD23, Agilent) with hydrogen gas. The data were analyzed with Agilent ChemStation software using the areas under the curve of each analyte.

Results

Composition of fatty acids

In all extracts, 5 fatty acids were detected: myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids; only in the hexane extract was detected the linolenic (18:3) acid.

In the hexane extract, the most abundant fatty acids were palmitic, oleic and linoleic acids, with an unsaturation index of 2,2 and 2,1 in the *H. capsulatum* environmental and clinical isolates, respectively. In the chloroform extract the most abundant fatty acids were palmitic, stearic, oleic and linoleic acids, with an unsaturation index of 1,8 and 1,3 in the *H. capsulatum* environmental and clinical, isolates respectively. In the isopropanol extract of the *H. capsulatum* environmental isolate, the most abundant fatty acids were palmitic, stearic, oleic and linoleic acids, with an unsaturation index of 1,6. In the isopropanol extract of the clinical isolate the results were different: Different to the clinic isolate, the most abundant fatty acids were palmitic, oleic and linoleic acids with an unsaturation index of 2,6.

In the methanol extract, the most abundant fatty acids were myristic, palmitic, stearic and oleic acids, with an unsaturation index of 0,6 and 0,7 in the *H. capsulatum* environmental and clinical isolates, respectively.

Conclusions

The proportion of unsaturated fatty acids found in the *H. capsulatum* environmental isolate decreases continuously as the polarity of the solvent increases. In the clinical isolate there is a large decrease in stearic acid in the extract with isopropanol, presenting significant differences in the index of unsaturation 2,6. These differences suggest that the *H. capsulatum* environmental isolate changes its relationship between the cell wall saturated and unsaturated fatty acids in order to be able adapting to the body temperature of the host

The comparative study of fatty acid composition of mycelial cell wall from environmental and clinical isolates of *H. capsulatum*, suggest a correlation between the lipid content and the virulence of this fungus.