PHYLOGENETIC CLASSIFICATION OF A COLOMBIAN BASIDIOMYCETE PRODUCER OF CYTOTOXIC COMPONENTS AGAINST JURKAT CELLS

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Introduction. Approximately 150,000 different mushrooms existing on Earth and probably less than 10% have been described (1). Although many morphological descriptions were made, phylogenetic analyses of rRNA gene sequences is one of the most used descriptive tools to understand the fungal taxonomy and diversity (2). For this purpose, regions of different fungal rRNA genes as internal transcribed spacer (ITS), small subunit (SSU) and large-subunits (LSU) have been reported (2). The Internal ITS regions of fungal ribosomal DNA (rDNA) are sequences with high variability, which allowed to distinguish fungal species. In this work, a phylogenetic analysis of the ITS region of a new Basidiomicete from Colombia was performed. This mushroom was morphologically classified as *Humphreya coffeata* (Berk.) Stey. (Ganodermataceae). The importance of this analysis lies is the molecular classification of this mushroom which is used as an alternative medicine. Also, previous data reports a cytotoxic activity on lymphoma cell line (Jurkat) by submerged culture filtrates (3).

Methods. The test sample was isolated from Tierra Alta, Colombia and acquired from the culture collection of the Universidad EAFIT (Medellin, Colombia). Fungus was grown on PDA medium and then in submerged cultures in shake flasks (glucose, 35.0; peptone, 5.0; yeast extract, 2.5 g/L) at 120 rpm, 30ºC, pH of 5.5 for 7 days. Genomic DNA was extracted from fungal tissue, using the Qiagen DNeasy Kit, using the manufacturer's protocol. From genomic templates, a portion of the ITS1, 5.8S and ITS2 gene fragment was amplified via PCR using the primers ITS1F, ITS4 and ITS4B previously described (4). Amplicons were sequenced on an ABI Prism 3100 capillary machine. Sequences were aligned, compared by BLAST and evolutionary analyses were conducted using MEGA version 5 (4).

Results. Tree sequences were amplified with ITS1F and ITS4 or ITS1F and ITS4B. Those sequences were aligned and positions with less than 50% site coverage were eliminated. A total of 596 positions were consistent in the final dataset, which were analyzed by BLAST. Eighteen sequences were selected for the phylogenetic analysis that was performed by Maximum Likelihood method. The bootstrap consensus tree was inferred from 1000 replicates (5). The percentage of replicate trees is shown next to the branches (5). The more related species were *Polyporales* sp, *Agaricomycetes* sp, *Irpex hydnoides* and *Basidiomycota sp*.

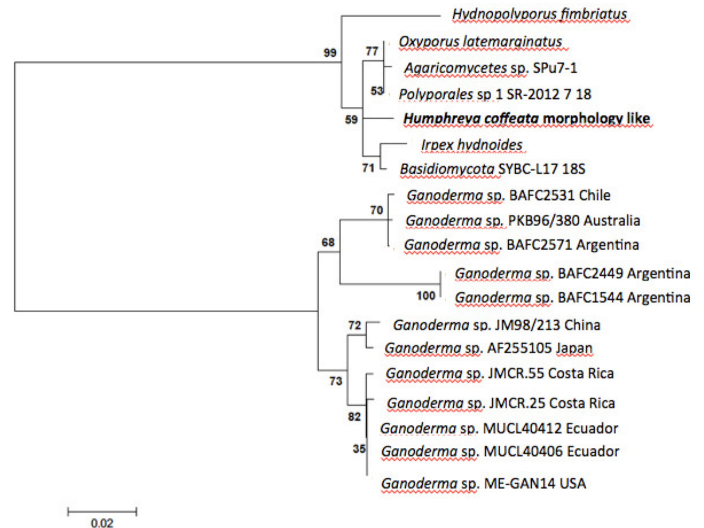

Fig.1. Maximum Likelihood phylogenetic tree, using ITS 1, ITS 2 and the 5.8 ribosomal subunit. The sequences have their identification number and the problem sequence was named as *Humphreya coffeata morphology like*.

Conclusions. Even the morphological characterization showed that the fungus under study belongs to specie *Humphreya coffeata* (Berk.) Stey. (Ganodermataceae), the molecular classification using ITS sequences shows that *Polypores* sp, *Agaricomycetes* sp, *Irpex hydnoides* and *Basidiomycota* are the more related species. Importantly, its morphology is different to those groups. So far, each of these groups has recognized orders, intermixed with all other fruiting body (basidiomes) types (6). Moreover, the problem sequence was separated importantly from *Ganoderma* species. All this analysis will serve to classify correctly the fungus named as "*Humphreya coffeata"* with medical importance.

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