ISOLATION AND IDENTIFICATION OF SYMBIOTIC FUNGI OF THE ANT *Atta mexicana* AND GLUCOSE DETERMINATION ACCUMULATED IN GONGYLIDIA

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Introduction. The ant *Atta mexicana* is recognized for their long lines of individual carrying small pieces of vegetable matter. The leaf-cutter ants, attacks a wide range of plants, causing losses in various crops. The plant material harvested by the ants is used to grow fungi, which provides enzymes that degrade plant polymers, releasing sugars that accumulate as glycogen into globular structures called gongylidia [1]. This ability could be used to recover sugar from cellulosic residues. Herein, this study was focused on the isolation and identification of symbiotic fungi of *Atta mexicana*, recover the glucose from gongylidia, and the determination of growth under solid-state culture in columns.

Methods. An inoculum of fungus, obtained from an *Atta mexicana* colony, was cultivated on potato dextrose agar (PDA), Malt Extract Agar (MEA), Wallerstein Differential Agar (WLDA) and Yeast Extract-Peptone-Glucose Broth (YPD), incubated at 25±2 and 30±1℃. Micrographs w ere obtained with a microscope Carl Zeiss. After 12 days the biomass produced in MEA was collected and freeze dried (MEA). Another biomass sample was frozen in liquid N_2, pulverized in a mortar and then freeze-dry (MEA N2). Also a fungal sample from the ant colony was freeze dried (Colony N2). Glucose extraction was tested for 1.5 h with 0.6M HCl at 98℃, H_2O at 98℃ or H_2O at 25℃ [2]. Glucose content was quantified in an YSI 2900 Biochemistry Analyzer. Genomic DNA was extracted from the isolated fungus and the primers ITS1 and ITS4 were used to amplify the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA). Sequences were analyzed with Blast at NCBI. Columns of 60 mL were packed with 1g of polyurethane foam; 2 g of leaves, mineral medium or malt extract and inoculated with the fungi. The columns were incubated at 26℃ with a bo ttom air flow at 200 mL/min. All analyses were performed by duplicate.

Results.
Growth was only observed in MEA and PDA at 25 ℃ after day 5. The micrographs showed an abundant mycelium formation and gongylidia accumulation of ~40µm. Pulverization and hydrolysis with HCl at 98 ℃, allowed the recovery 122 mgGlucose/gBiomass which was the best extraction condition of those tested. The sample from the ant colony contained only 20mgGlucose/gBiomass, however it should be noted that this sample contained not only fungal biomass but also some vegetal material. BLAST analysis of the PCR amplified ITS showed a 98% sequence homology with *Leucoagaricus gongylophorus*. After 7 days of culture growth was observed in all the columns although it was higher with malt extract.

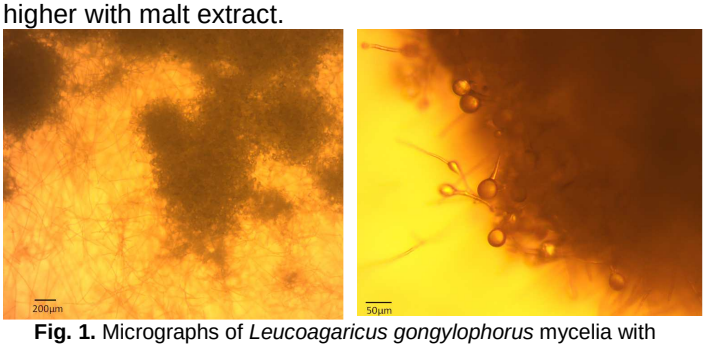

Fig. 1. Micrographs of *Leucoagaricus gongylophorus* mycelia with gongylidia accumulation.

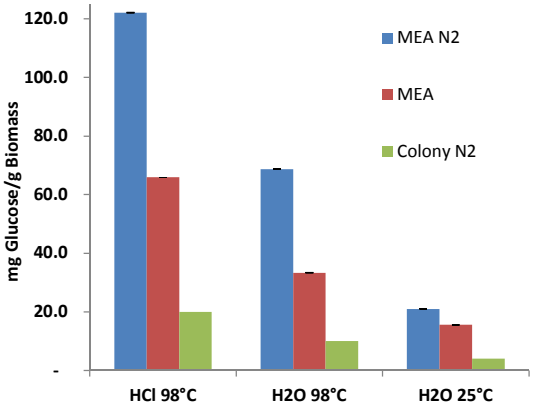

Fig. 2. Treatments to release glucose accumulated in gongylidia.

Conclusions.
To the best of our knowledge, this is the first time that the symbiotic fungi *L. gongylophorus* was isolated and identified from *Atta mexicana.* The fungus is able to grow on plant leafs and accumulate glycogen in gongylidia, which could be used to obtain fermentable sugars from lignocellulosic residues.

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