Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

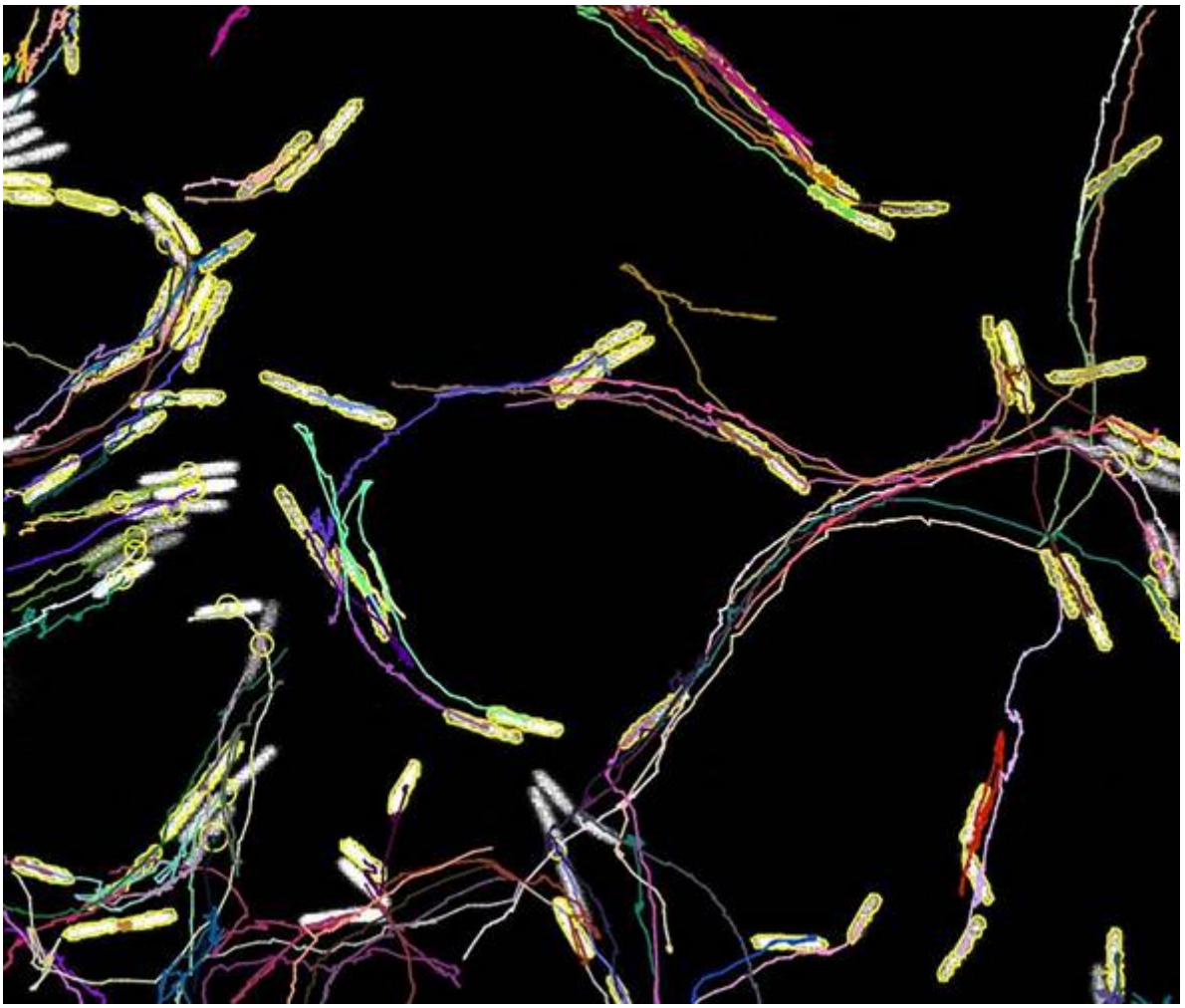

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

CHARACTERIZATION OF CONSERVED ENZYMES FROM *Auricularia fuscosuccinea* LBM 244 BY SECRETOME ANALYSIS

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Auricularia fuscosuccinea LBM 244 is an edible fungus that produces and secretes hydrolitic enzymes that can be used in the green enzyme-assisted extraction of biological compounds from vegetable cell walls. Cellulases and hemicellulases are the principal enzymes used in this bioprocess. These enzymes present conserved regions that enable the amplification of their coding genes for future bioengineering applications. This work aimed to identify and characterize the enzymes secreted by *A. fuscosuccinea* LBM 244 and amplify the gene region of the most representative enzyme of the fungal secretomes. To obtain an enzymatic cocktail with cellulolytic activity, *A. fuscosuccinea* LBM 244 was grown in Czapek minimal medium supplemented with the agro-industrial wastes sugarcane bagasse, cassava bagasse or jabuticaba peels. The fungus was inoculated in an unsupplemented medium and a medium supplemented with glucose as a control. Mass spectrometry was used to identify the extracellular proteins secreted by the fungus in the different media and the protein with the cellulolytic activity was selected. Protein identification was performed using Proteome Discoverer (Thermo Scientific) version 2.2 with the following database: *A. subglabra*, *Auricularia* sp., and *Agaricomyecetes*, since the genome of *A. fuscosuccinea* was not sequenced. Based on the amino acid sequence of the selected protein, a bioinformatic analysis was performed. For that, the T-Coffee Sequence Alignment program was used to analyze the conserved domains of the aminoacidic sequences of *Auriculariales* sp., *Auricularia subglabra* and *Exidia glandulosa* available in the GenBank database (NCBI). These regions were used to design the degenerate primers and thermodynamic properties were evaluated using the FastPCR program. These primers were used for PCR of the DNA extracted from *A. fuscosuccinea* LBM 244. For the visualization of DNA and PCR products, electrophoresis on agarose gels (1% and 0.1%, respectively) was performed. The secretome of the fungus grown on jabuticaba peels presented the highest number of proteins, followed by the secretome of the fungus grown on sugarcane bagasse. No proteins were identified in the secretomes of the fungus grown in cassava bagasee and the control media. The

only enzyme with cellulolytic activity was the endo-1,3(4)-?-glucanase found in the secretome of the fungus grown on jabuticaba peels. For this reason, this protein was selected to design the degenerate primers through its amino acidic sequence. Secretomic data were validated with a biochemical determination of the enzymatic activity. Several primers were designed and analyzed *in silico*. There were no introns in the sequence. MFF1 and MFR3 primers were selected based on their better thermodynamic properties and a 700 bp of the gene region was amplified. Endo-1,3(4)-?-glucanase is involved in the hydrolysis of the ?-glucan to glucose, which suggests this cocktail can be effectively used in enzyme-assisted extraction.

Palabras clave: Secretome-PCR-jabuticaba peels-Endo1,3(4)? glucanase-Auricularia fuscosuccinea