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EXPLORING FUNGAL BIODIVERSITY IN BRAZIL FOR NATURAL COLORANTS PRODUCTION

SAMPAIO, Bruno Leite¹

Doctor in Sciences, Natural and Synthetic Products

MIDORIKAWA, Gláucia Emy Okida²

Doctor in Molecular Biology

SILVA, Carolina Assunção Lucas³

Graduate in Pharmacy

SANTOS, Larissa Carmona Zonta⁴

Bachelor in Pharmacy, Cosmetology

MOLINARI, Patrícia Abrão de Oliveira⁵

Doctor in Sciences, Chemistry

ABSTRACT

An alternative route for the sustainable production of natural colorants with potential use in cosmetics, food, feed, paint, and textile industries is the biotechnological route. Fungi are a rich source of biologically active natural compounds. The Brazilian fungal biodiversity can be rationally explored to produce known or novel colorants. The objectives of this study were to select novel pigment-producing fungal strains, characterize the major colored metabolites in the selected crude extracts, and evaluate the extracts for their stability to light, pH, and temperature. A total of 229 fungal strains isolated from plants and soils of the Amazon, Cerrado, and Atlantic Forest biomes were kindly provided by the “Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries” (CMMAABio) of Embrapa Agroenergia. In primary screening of the 229 fungi, the strains demonstrating visual colorant production on PDA, MEA, and YES plates (incubated at 25°C in the absence of light, and evaluated at 7, 14, and 21 days after inoculation) were selected. The 32 selected strains were evaluated in a secondary screening (25°C in the absence of light, 10 days, 180 rpm) in PDB, MEB, and Czapek under submerged fermentation conditions in shake flasks. All tests were performed in triplicate. The strains named EF1, EF2, EF3, and EF4 were selected for further characterization because they produced intensely colored supernatants (pink, purple, orange, and red). The major colored metabolites in the selected supernatants (crude extract lyophilized) were identified (chemical class) using a combination of chromatographic and mass spectrometry-based techniques (LC-

¹ Embrapa Agroenergia, Brasília, Federal District, e-mail bruno.farmueg@gmail.com

² Embrapa Agroenergia, Brasília, Federal District, e-mail glauciaemy@gmail.com

³ Embrapa Agroenergia, Brasília, Federal District, e-mail carolina.als94@yahoo.com

⁴ Grupo Boticário, São José dos Pinhais, Paraná, e-mail larissa.zonta@grupoboticario.com.br

⁵ Embrapa Agroenergia, Brasília, Federal District, e-mail patricia.oliveira@embrapa.br

MS, LC-MS/MS, and UPLC-DAD). The analysis of the EF1 sample identified 12 metabolites, of which nine are known azaphilones (two orange and seven yellow). Six known metabolites were identified in the EF2 sample, all described for the first time in this fungal species (some quinones with red color, and other yellow and brown secondary metabolites). In the EF3 sample, seven metabolites were identified, of which five are known red naphthoquinones. In the sample EF4, three metabolites were identified; the major colored metabolite is a previously described anthraquinone with intense red color, which is described for the first time in this fungal species. Accelerated stability experiments were carried out under light (λ_{\max}) and temperature of 50°C at different pHs (pH 4.6, 5.5, and 7.0) using the butanolic extracts from the selected fungi supernatants. After seven days of exposure to light (λ_{\max}) and temperature of 50°C, the percentage of degradation of the colorants at different pHs varied from 35% to 55% for the EF1 extract, from 65% to 74% for EF2, from 31% to 43% for EF3, and from 26% to 55% for EF4. The butanolic extracts of the selected fungi supernatants have colorants with chemical structures stable to light and temperature. Interestingly, the selected fungi belong to taxonomic groups that have not been exploited for industrial colorant production, indicating that fungal biodiversity is a prolific source of new strains for natural colorants production.

Keyword: fungal biodiversity, natural colorants, light stability, anthraquinone, submerged fermentation

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