NEW YEAST STRAINS FROM THE BRAZILIAN CERRADO BIOME FOR THE PRODUCTION OF CAROTENOIDS

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ABSTRACT

Carotenoids are pigments responsible for the colors yellow, orange, red, and purple in a wide variety of plants, algae, fungi, bacteria, and archaea. They are widely applied in the food, feed, medical, pharmaceutical, and cosmetic industries as colorants and functional ingredients. However, chemical synthesis accounts for most of the commercial carotenoids while there is a high market demand for natural carotenoids. Yeasts are a rich source of biologically active carotenoids. The Brazilian microbial biodiversity can be rationally explored for the production of either existing or novel carotenoids. The objectives of this study were to select novel carotenoids-producing yeast strains and characterize the major colored metabolites in the selected extracts. A total of 64 yeast strains isolated from plants of the Brazilian Cerrado biome were kindly provided by the "Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries" (CMMAABio) of Embrapa Agroenergia. In primary screening, the strains demonstrating fast growth and intensely colored colonies on PDA and YPDA plates (28°C in the absence of light, and analysis at 3 and 7 days after inoculation) were selected. Six selected strains were evaluated in a secondary screening in PDB under submerged fermentation conditions in shake flasks (28°C in the absence of light, 72 hours, 180 rpm). All tests were performed in triplicate. The strains named 70A and 108 were both identified as *Sporobolomyces pararoseus* and were selected for further characterization because they presented intensely colored cells (orange) and high cell density under the conditions used. For carotenoid extraction, each strain was cultured

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in PDB (1 L) in the absence of light at 28°C, 200 rpm for 72 hours. Pelleted cells were grounded to a fine powder in liquid nitrogen and mixed with dimethyl sulfoxide. After incubation in a water bath, a solution containing acetone and methanol was added to the samples. Then they were centrifuged and further evaporated at 35ºC, thus resulting in DMSO extracts. Subsequent partitions were performed with hexane and ethyl acetate and these fractions were evaporated at 40ºC. The major colored metabolites in the hexane and the ethyl acetate fractions from 70A and 108 strains were identified (chemical class) using a combination of chromatography and mass spectrometry-based techniques (LC-MS, LC-MS/MS, and UPLC-DAD). In the hexane fractions, β-carotene and torulene were detected as significant compounds. Besides, other substances of lipidic nature were identified in both fractions (p. ex.: fatty acid amides, monoacylglycerol, diacylglycerol, triacylglycerol, and phospholipids). Also, other bioactive metabolites were identified in the evaluated samples, such as oleamide, p-coumaric acid, and diketopiperazines. The results indicate that fungal biodiversity from the Brazilian Cerrado biome is a prolific source of new yeast strains for carotenoids and bioactive metabolites production in the bioeconomy context.

Keyword: *Sporobolomyces pararoseus*, β-carotene, torulene, submerged fermentation, biodiversity

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