Evaluation of carotenoid production by *Rhodotorula glutinis* strains isolated from the Brazilian Atlantic Forest using pentoses as a carbon source.

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ABSTRACT

Currently, biopigments produced by microorganisms have stood out compared to synthetic pigments, mainly due to their favorable properties for human health and for the environment. The yeast *Rhodotorula glutinis* has been evaluated due to its capacity of producing carotenoids using alternative carbon sources. Their ability to metabolize pentoses is a relevant characteristic from the viewpoint of using hemicellulosic fraction of lignocellulosics, as C5 sugars are abundant carbohydrates that are not metabolized by most microorganisms. In this context, four wild strains of *Rhodotorula glutinis*, UFMG-CM-Y5406, UFMG-CM-Y5408, UFMG-CM-Y5409 and UFMG-CM-Y5412, isolated from the Brazilian Atlantic Forest, were evaluated regarding to their potential to produce carotenoids using xylose and arabinose as substrates. Cell growth, substrate consumption and carotenoid production were evaluated. The results obtained showed the potential of the wild yeasts. Production was favored in xylose-based media, and the best results were obtained using the yeast *Rhodotorula glutinis* UFMG-CM-Y5408, which produced 57 µg/g of carotenoids.

Keywords: Carotenoids, *Rhodotorula glutinis*, xylose, arabinose

1 INTRODUCTION

In recent years, the market has seeking for reducing the use of synthetic products, replacing them with natural products that bring health benefits. In this context, microbial biopigments have stood out in relation to synthetic pigments, since, in addition to providing color, they also have nutraceutical potential and are environmentally friendly (MUSSAGY; RIBEIRO; PEREIRA, 2023).

Despite these advantages, it is still a challenge to make the process of industrial production of microbial biopigments economically competitive, mainly due to the high cost of the components of the growing medium. One alternative that has been adopted is the use of agro-industrial waste and by-products as a source of nutrients for fermentation (DE MEDEIROS; DUFOSSÉ; BICAS, 2022). The study of processes capable of making use of all fractions of the raw material is essential for making bioprocesses viable, by means of new technological routes to obtain a greater variety of products. The hemicellulose fraction, which can account for a considerable percentage of various lignocellulosic materials, is mainly composed of pentoses, and its application in bioprocesses is still limited compared to cellulose, due to the inability of many microorganisms to ferment pentoses (CHANDUKISHORE et al., 2024).

In this context, it is interesting to use microbial strains capable of metabolizing pentoses, such as *Rhodotorula glutinis* yeasts, which metabolize various types of substrates, including alternatives derived from agro-industrial waste and by-products. This yeast is tolerant to a wide temperature and pH range and their metabolic pathways are versatile, presenting an enzymatic complex capable of producing various bioproducts (MUSSAGY; RIBEIRO; PEREIRA, 2023). Among the bioproducts, carotenoids biopigments are recognized for their strong antioxidant, anti-inflammatory, and anti-cancer activities, as well as acting in the prevention of cardiovascular diseases and sun protection (RAPOPORT et al., 2021).

The aim of this study was to evaluate the production of carotenoid biopigments by four strains of *R. glutinis*, isolated from the Brazilian Atlantic Forest, using the main pentoses present in lignocellulosic materials, xylose and arabinose, as carbon sources.

2 MATERIAL & METHODS

Four strains of *Rhodotorula glutinis* belonging to the Microorganism Collection of the Federal University of Minas Gerais (UFMG) were evaluated: UFMG-CM-Y5406, UFMG-CM-Y5408, UFMG-CM-Y5409, and UFMG-CM-Y5412. The yeasts were isolated from the nectar of *Ipomoea cairica* flowers in a conserved fraction of the Brazilian Atlantic Forest within the Rio Doce State Park, in the state of Minas Gerais, municipality of Marliéria, MG, Brazil.

The strains, preserved in Yeast-Malt-Agar (YMA) medium at 4°C, were used to prepare the inoculum in 125 mL Erlenmeyer flasks with 50 mL of medium containing 15 g/L of sugar (xylose or arabinose, correspondent to the sugar which was used in fermentation cultures), 2.5 g/L of yeast extract, 2 g/L of malt extract, 1 g/L of $(NH_4)_2SO_4$, 1 g/L of KH_2PO_4 and 0.25 g/L of $MgSO_4$ (AKSU; EREN, 2007). The culture was incubated in a rotary shaker at 150 rpm, 30°C, for 48 hours. After this period, the medium was centrifuged (3500 rpm, 20 min) and the precipitate was washed with sterile water. The cells were resuspended in sterile water and the biomass concentration was measured to inoculate adequate volumes to obtain the initial concentration of 1 OD in the fermentation experiments.
The fermentation cultures were carried out in triplicate for each of the *R. glutinis* strains in 125 mL Erlenmeyer flasks with 50 mL of medium. Cultivation media were formulated using 30 g/L of xylose or arabinose as a carbon source, 2.5 g/L of yeast extract, 2 g/L of malt extract, 1 g/L of $(NH_4)_2SO_4$, 1 g/L of KH_2PO_4 and 0.25 g/L of $MgSO_4$ (AKSU; EREN, 2007). The tests were carried out for 72 hours on a rotary shaker at 150 rpm, 30°C, with periodic sampling to quantify cell biomass using a UV-Vis spectrophotometer at 600 nm and substrate concentration using the DNS method for determining reducing sugars (MILLER, 1959; THUMKASEM et

al., 2023). At the end of 72 hours, the biopigments were extracted by breaking the cell using glass beads, suspended in acetone and petroleum ether and quantified by spectrophotometry at 450 nm, according to Ribeiro et al. (2019). They were also characterized by FTIR using a Cary 630 FTIR (Agilent, USA) equipped with an Attenuated Total Reflection accessory (ATR). The FTIR technique was conducted with a diamond crystal as the internal reflection element, applying 32 scans at a 8 cm^{-1} resolution using an spectral range of 4000 to 650 cm^{-1}.

3 RESULTS & DISCUSSION

The results obtained for microbial growth and consumption of xylose and arabinose by the four *R. glutins* yeast strains are shown in Figure 1.

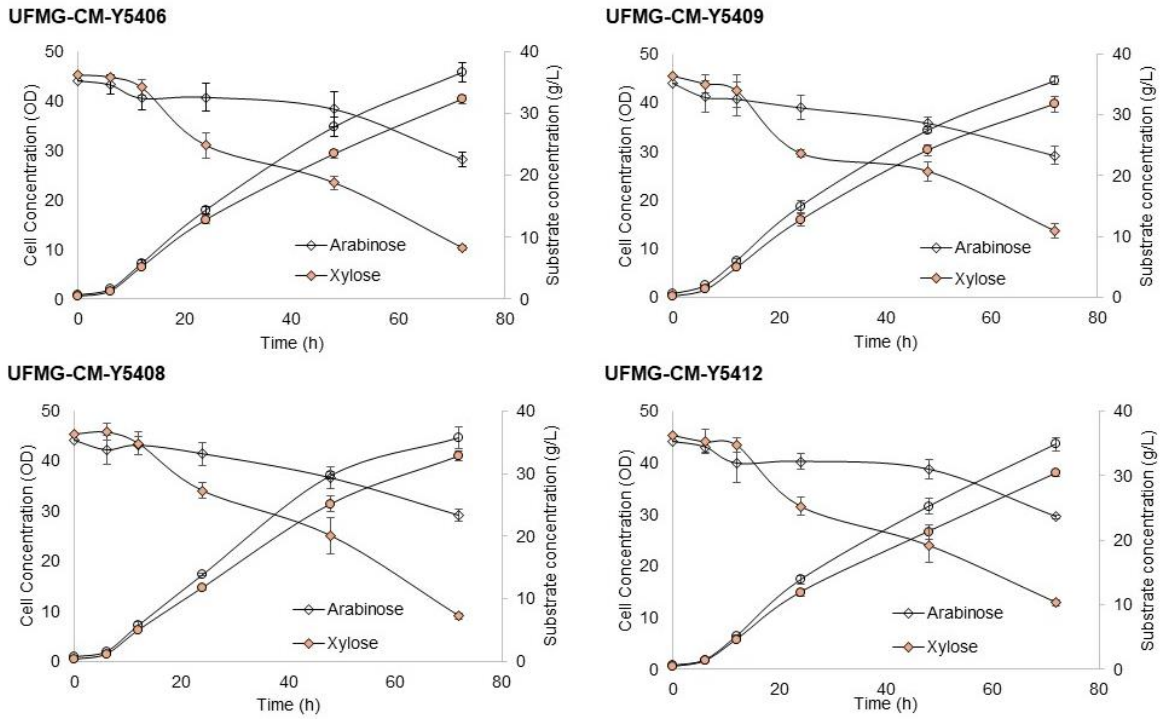

Figure 1: Cell growth and substrate consumption by different strains of *R. glutinis* in xylose and arabinose-based media. (-◊-) arabinose consumption (-♦-) xylose consumption (-○-) cell growth in arabinose-based medium (-●-) cell growth in xylose-based medium.

The results obtained for cell growth were similar for the four *R. glutinis* strains evaluated. In all cases, the final cell concentration was around 40 OD in the xylose-based medium and 45 OD in the arabinose-based medium. About substrate consumption, for the four yeast strains, xylose consumption was twice as high as arabinose consumption. After 72 hours of fermentation, 72% of the xylose had been consumed and only 34% of the arabinose.

The production of carotenoids, measured after 72 hours of cultivation, is shown in Figure 2.

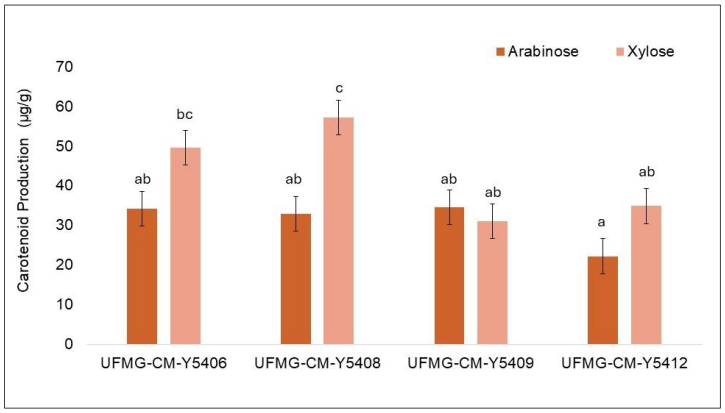

Figure 2: Production of carotenoids by different strains of *R. glutinis* in media based on xylose and arabinose.

The production of carotenoid biopigments by the four *R. glutinis* yeast strains evaluated was better in a xylose-based medium. The best results were obtained using the UFMG-CM-Y5408 strain with a production of 57 µg/g, about 2 times more than that obtained by the same strain in arabinose-based medium.

A preliminary characterization of the carotenoids was carried out by FTIR, and the results are shown in Figure 3.

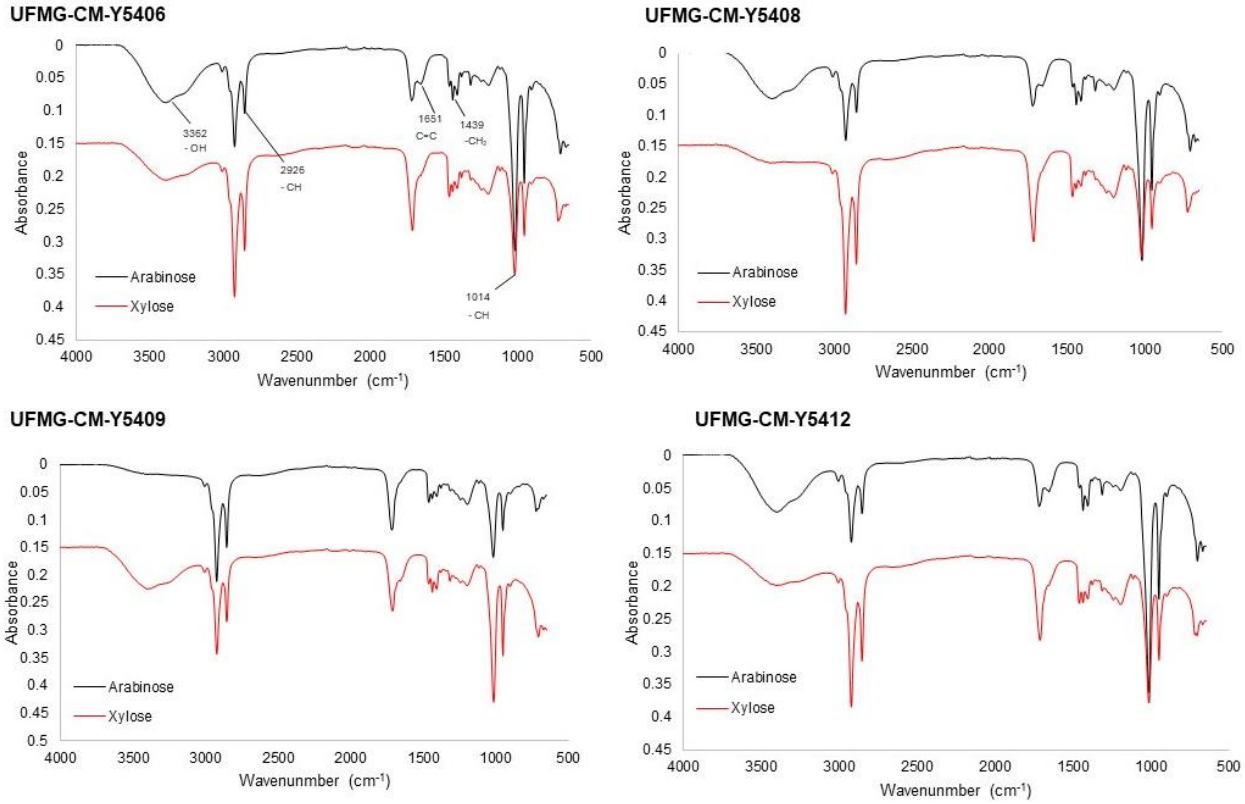

Figure 3: FTIR spectrum of carotenoids produced by R. glutinis yeasts in xylose and arabinose-based media.

The FTIR spectra obtained showed similar profiles for the four yeast strains, regardless of the substrate used. Although there were small differences, especially in the intensity of the peak at 3362 cm^{-1} corresponding to stretching vibration of the hydroxyl functional group, indicating a possible variation in the composition of carotenoids produced according to the strain and substrate used. In all cases, typical carotenoid spectra were obtained, containing peaks around 1600 cm^{-1} indicating the stretching vibration of unsaturation (C=C) and peaks between 2800 – 3000 cm^{-1} typical of bending vibration of CH_2 groups.

4 CONCLUSION

The results obtained show the potential for carotenoid production by *R. glutinis* yeasts using pentoses as carbon sources. The cell growth and substrate consumption profiles were similar for the four yeast strains evaluated. However, carotenoid production was more significant using the yeast *R. glutinis* UFMG-CM-Y5408 in xylose-based culture medium.

REFERENCES

AKSU, Z.; EREN, A. T. Production of carotenoids by the isolated yeast of Rhodotorula glutinis. **Biochemical Engineering Journal**, v. 35, n. 2, p. 107–113, 2007.

DE MEDEIROS, T. D. M.; DUFOSSÉ, L.; BICAS, J. L. Lignocellulosic substrates as starting materials for the production of bioactive biopigments. **Food Chemistry: X**, v. 13, n. November 2021, 2022.

MILLER, G. L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. **Analytical Chemistry**, v. 31, n. 3, p. 426–428, 1959.

MUSSAGY, C. U.; RIBEIRO, H. F.; PEREIRA, J. F. B. **Rhodotorula sp. as a cell factory for production of valuable biomolecules**. [s.l.] Elsevier, 2023. v. 123

RAPOPORT, A. et al. Carotenoids and some other pigments from fungi and yeasts. **Metabolites**, v. 11, n. 2, p. 1–17, 2021.

SANTOS RIBEIRO, J. E. et al. Rhodotorula glutinis cultivation on cassava wastewater for carotenoids and fatty acids generation. **Biocatalysis and Agricultural Biotechnology**, v. 22, n. October, 2019.

CHANDUKISHORE, T. et al. Re-routing the hemicellulosic fraction of lignocellulosic biomass toward value added products: A pragmatic bio refinery approach. **Journal of Environmental Chemical Engineering**, v. 12, n. 2, p. 111971, 2024.

THUMKASEM, N. et al. Rhodotorula paludigena CM33 cultivation process development for high β-carotene single cell protein production. **Biocatalysis and Agricultural Biotechnology**, v. 54, n. July, p. 102926, 2023.

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