Potential of wild strains of *Rhodotorula glutinis* isolated from preserved Atlantic Forest fragments and the use of glycerol as an alternative substrate for biopigments production

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ABSTRACT

Bioprospecting new microbial isolates from Brazilian biomes can uncover new potentials in the nation's genetic heritage, contributing to the production of value-added bioproducts such as biopigments. In this scenario, the aim of this study was to assess the potential for biopigment production using four different strains of *Rodothorula glutinis* isolated from the Brazilian Atlantic Forest. Two different carbon sources, glucose and glycerol, were evaluated in submerged culture for the four yeast strains. After 72 hours of cultivation, all strains showed similar growth profiles and substrate consumption, with 50% and 33% consumption of glucose and glycerol, respectively. The strain *R. glutinis* UFMG-CM-5408 yielded the highest total carotenoid productions, with average productions of 97.84 µg/g and 109.9 µg/g in glucose and glycerol-based media, respectively. These carotenoids exhibited similar chemical profiles, as evidenced by FTIR spectrum analysis. This study highlights the biotechnological potential of *R. glutinis* strains for carotenoid production using alternative carbon sources such as glycerol.

Keywords: carotenoids, biodiversity, biodiesel by-products, fungal colorants.

1 INTRODUCTION

The prospecting of new microbial strains in underexplored biomass and substrates enables the valorization of important natural resources, such as flora, fauna, and specific ecosystems. An example of a significant Brazilian biome is the Atlantic Forest, considered the most threatened biome in Brazil. It currently covers only about 5% of its original territory, most of which is located within specific regions, conservation parks, and private reserves. The Atlantic Forest is one of the biomes with the highest species biodiversity, including microbial biodiversity with significant yet underexplored biotechnological potential[1].

Wild yeast strains, including species of *Rhodotorula glutinis*, are still underexplored and have great potential for studies to obtain bioproducts, including biopigments (carotenoids), biomolecules that hold significant market value and are gaining prominence in the functional products sector. Besides their coloring properties, other additional characteristics, such as proven antimicrobial, antioxidant, and anticancer, make these biopigments attractive for various industrial sectors, including the food, pharmaceutical, and cosmetic industries[2].

However, a limitation in the production of carotenoids via microbial pathways is the production costs. The formulation of low-cost culture media can be interesting to reduce expenses and enable efficient conversion metabolism into products. Glycerol, a by-product in biodiesel production, emerges as an alternative carbon source for the production of carotenoids[3].

Therefore, the aim of this study was to explore the potential of cell growth, substrate consumption, and carotenoid production, in submerged culture, of four different wild strains of *Rhodotorula glutinis* isolated from the nectar of *Ipomoea cairica* in the Atlantic Forest biome, in two different culture media, based on glucose and based on glycerol.

2 MATERIAL & METHODS

Four different wild strains of *R. glutinis* were used: UFMG-CM-Y5406, UFMG-CM-Y5408, UFMG-CM-Y5409, and UFMG-CM-Y5412. They were provided and belonging to the Collection of Microorganisms, DNA and Cells of Universidade Federal de Minas Gerais (UFMG). The yeast strains were isolated from the nectar of flowers of *Ipomoea cairica* within the Rio Doce State Park, considered the largest preserved fragment of Atlantic Forest in the state of Minas Gerais, municipality of Marliéria, MG, Brazil. The different strains of *R. glutinis* were streaked onto YMA (Yeast, Malt, Agar) plates and remain stored at 4 ºC for further use.

The inoculum, for all cases, was prepared in 125 mL Erlenmeyer flasks containing 50 mL of culture medium composed[4] of glucose (15 g/L), yeast extract (2.5 g/L), malt extract (2.5 g/L), $(NH_4)_2SO_4$ (1 g/L), KH_2PO_4 (1 g/L), and $MgSO_4$ (0.25 g/L), and incubated at 30°C on a shaker-type incubator for 48 hours. After the inoculum growth, the cells were centrifuged (2450 *xg* for 20 min) and washed twice with distilled water, separately for each strain of *R. glutinis.*

The cultures using glucose and those using glycerol as a carbon source were conducted in triplicate in 125 mL Erlenmeyer flasks, each containing 50 mL of culture medium composed of 30 g/L of the respective carbon source (glucose or glycerol) and supplemented with nitrogen sources and salts as described for the inoculum preparation. The cultivation was carried out under the same temperature and agitation conditions as the inoculum preparation, for 72 hours, starting with an initial optical density (OD) of 1.0 obtained from the inoculum. Throughout all cultures, periodic analyses were performed to quantify cell growth and substrate consumption, and at the end of the cultivation, the production of total carotenoids was determined.

Cell growth was determined by optical density in a spectrophotometer (OD_{600nm})[5]. Glucose was determined using the colorimetric method for total reducing sugars, using 3,5-dinitrosalicylic acid (DNS), and the concentration of reducing sugars was determined by spectrophotometry at 540 nm, with values compared to a previously determined calibration curve. Glycerol quantification was determined by High Performance Liquid Chromatography (HPLC) on a Bio-Rad (Hercules, CA) Aminex HPX-87H column (300 × 7.8 mm) at 45°C with H_2SO_4 (5 mM) as the eluent, a flow rate of 0.6 mL/min, and a sample volume of 20 µL. Total carotenoids quantification was performed as proposed by Ribeiro et al.[6], in which cells were disrupted by freeze/thaw cycles using glass beads suspended in DMSO, acetone, and petroleum ether, and solubilized carotenoids were quantified by spectrophotometry at 450 nm.

The chemical profile of carotenoids was evaluated using by Fourier transform infrared, 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 with 8 cm^{-1} resolution, using an spectral range of 4000 to 650 cm^{-1}.

3 RESULTS & DISCUSSION

The macroscopic aspects of the colonies of the studied *R. glutinis* strains are presented in Figure 1. All yeast colonies exhibit a smooth appearance, regular colony edge, and coloration ranging from dark yellow to shades of orange.

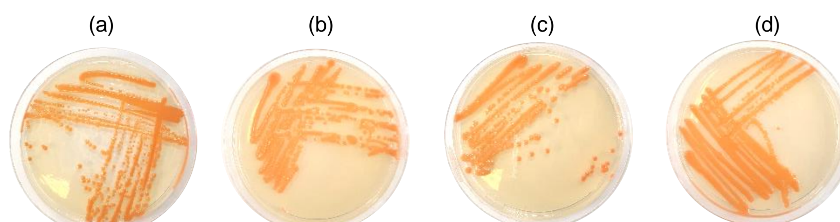

Figure 1: Photograph of *R. glutinis* colonies on YMA plates after 48 hours of growth. (a) UFMG-CM-Y5406, (b) UFMG-CM-Y5408, (c) UFMG-CM-Y5409, and (d) UFMG-CM-Y5412.

The results of cell growth in the two different formulated media, as well as the substrate concentrations, are compiled and presented in Figure 2.

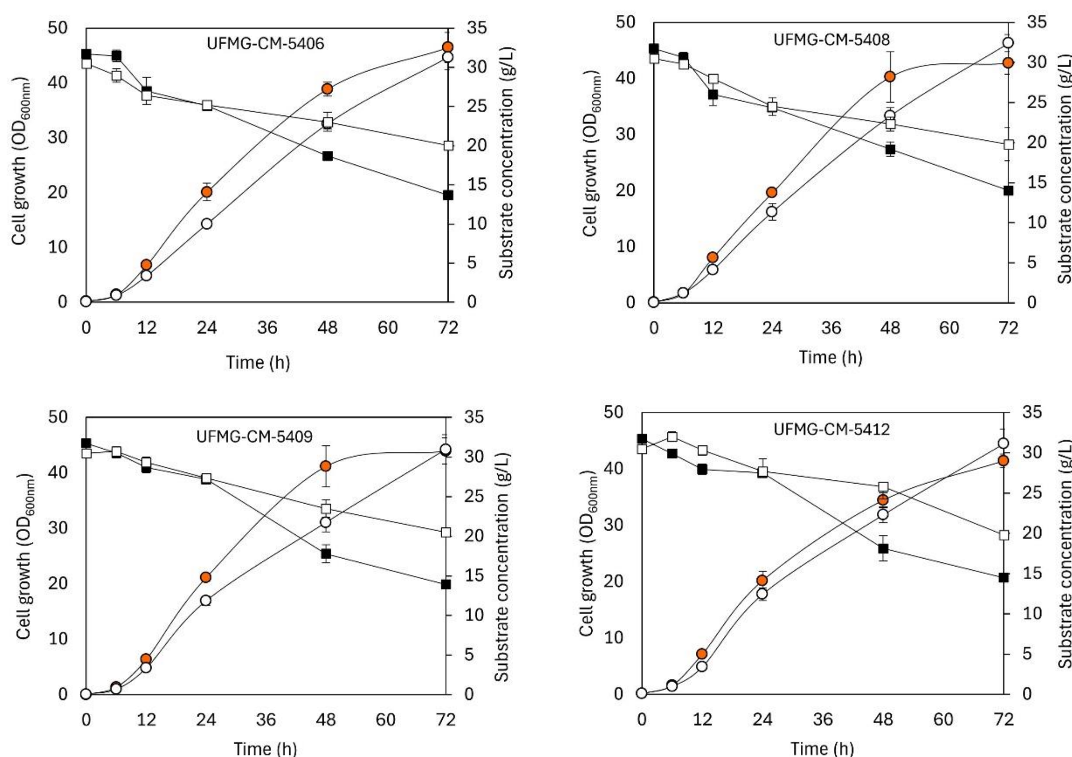

Figure 2 Cell growth and substrate consumption in submerged culture of different wild strains of R. glutinis. (-●-) Cell growth in medium formulated with glucose, (-○-) cell growth in medium formulated with glycerol, (-■-) glucose concentration, and (-□-) glycerol concentration.

The four different strains of *R. glutinis* showed a very similar cell growth profile, even when submitted to different culture media. When comparing the two different culture media, the medium formulated with glucose, as a whole, resulted in a little faster growth when compared to the medium formulated with glycerol. After 72 hours of cultivation, only the UFMG-CM-Y5408 and UFMG-CM-Y5412 strains showed greater OD_{600} in glycerol.

The substrate consumption profile showed similar consumption of glucose and glycerol in the first 24 hours of cultivation. However, from 48 hours, the consumption and metabolization of glucose was faster when compared to the consumption of glycerol, a behavior observed in the four *R. glutinis* strains evaluated. After 72 hours of cultivation, about 50% consumption of glucose and 33% consumption of glycerol was observed.

The production of total carotenoids, determined at the end of each cultivation, is shown in Figure 3.

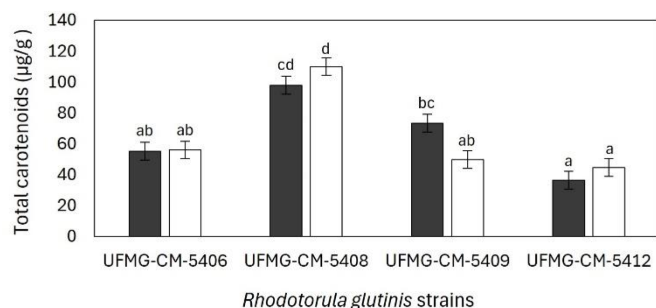

Figure 3 Production of total carotenoids using different wild *R. glutinis* strains, with glucose and glycerol as substrates. Dark bars: medium formulated with glucose; White bars: medium formulated with glycerol. Note: Mean values followed by the same letters do not differ by Tukey's test ($p>0.05$).

Although presenting similar growth profiles and substrate consumption, the production of total carotenoids differed by each used microorganism. Strain UFMG-CM-5408 promoted the highest carotenoid productions with both evaluated substrates, with an average production of 97.84 µg/g and 109.9 µg/g in glucose and glycerol-based media, respectively, a value about 2.6x and 2.4x higher when compared to the *R. glutinis* UFMG-CM-5412, strain with lower production under the same conditions.

The carotenoids produced by *R. glutinis* UFMG-CM-Y5408 were partially characterized by FTIR, and the spectra are shown in Figure 4.

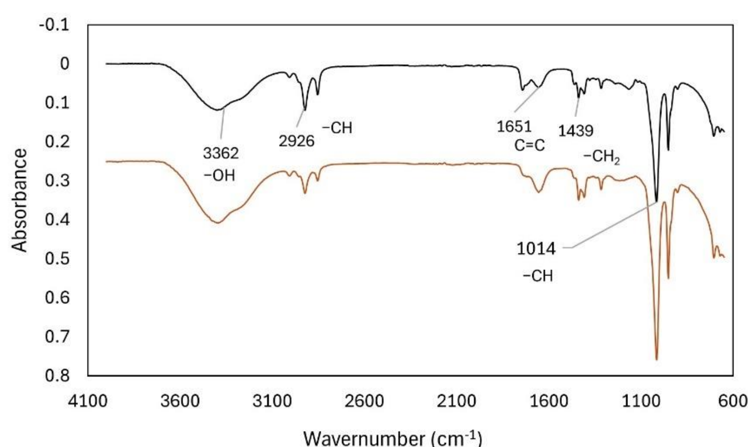

Figure 4 FTIR spectrum of carotenoids obtained from the growth of *R. glutinis* UFMG-CM-Y5408. Spectrum with black lines: medium formulated with glucose; Spectrum with orange lines: medium formulated with glycerol.

The FTIR spectra exhibit typical functional groups of olefinic hydrocarbons, with specific branching regions containing hydroxyl groups (3362 cm^{-1}), regions of unsaturation such as C=C bonds (1651 cm^{-1}), and CH and CH_2 groups (1014 and 1439 cm^{-1}). Figure 4 depicts similar chemical profiles, even when different carbon sources are used for production and consumption, highlighting the versatility of the strain UFMG-CM-Y5408 in producing similar bioproducts, despite varying carbon sources.

4 CONCLUSION

This study showcased the growth, consumption, and biopigment production potential of four different wild strains of *R. glutinis*, as well as the potential use of glycerol as a carbon source. Among the different strains studied, *R. glutinis* UFMG-CM-Y5408 yielded the highest total carotenoid productions, in media formulated with glucose or glycerol. The findings presented reveal the potential of unexplored wild strains, thereby stimulating bioprospecting studies and the development of new products.

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