ANALYZING THE GROWTH PROFILE OF YEASTS ISOLATED FROM ARAUCARIA FOREST IN SOUTHERN BRAZIL

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ABSTRACT

The diverse yeast populations in Brazil, spanning six distinct biomes, play crucial roles in nutrient cycling and symbiotic relationships within ecosystems. Studying these microorganisms is essential for understanding species distribution and their potential biotechnological applications. The expanding use of yeasts in biotechnology, from food production to biofuels and pharmaceuticals, underscores the need to explore yeast biodiversity for sustainable bioproduct production. This study focused on isolating and evaluating yeast strains from the Araucaria Forest to enhance waste exploitation and treatment processes. Samples from the Chapecó National Forest were collected, isolating 32 yeast strains from soil, bark, and leaf litter. To assess their growth rate, these isolates were cultured in eight different carbohydrates related to vegetal residues. Most isolates grew on multiple carbohydrates, with some showing specific affinities. Notably, strains CHAP-265 and CHAP-267 showed the highest growth rates in xylose and cellobiose, while CHAP-261 excelled in arabinose utilization, indicating potential for producing bioproducts like arabitol and ethanol. This research highlights the importance of exploring biodiversity to find yeast strains suitable for bioprocesses utilizing plant residues as substrates, promoting sustainable production methods.

Keywords: Araucaria Forest. Prospecting yeast. Xylose, Arabinose. Celobiose

1 INTRODUCTION

The diversity of yeasts in Brazil represents a field to be better explored, considering the vast territorial extent of the country, which has six biomes with distinct characteristics[1,2]. Within these biomes, yeasts play fundamental roles in ecosystems, actively participating in nutrient cycling and symbiotically interacting with other organisms[3,4]. In this context, studying the biodiversity of these microorganisms in different Brazilian biomes is crucial for understanding species distribution and their potential biotechnological applications.

Yeasts isolated from plants in the Cerrado and from rotten wood samples in biomes such as the Amazon, Cerrado, and Atlantic Forest demonstrate the presence of a wide variety of species in different environments — many poorly characterized[1]. One of the vegetation types that make up one of these biomes is the Mixed Ombrophilous Forest (MOF), also known as the Araucaria Forest, which spans the states of Paraná, Rio Grande do Sul, and Santa Catarina[5]. Unlike the vegetations that comprise the southeast of the country, the diversity of yeasts in the MOF needs to be better explored.

The use of yeasts in biotechnology has expanded beyond the traditional production of foods such as bread and alcoholic beverages to areas like biofuel and pharmaceutical production. In view of this expansion, plant residues can be used as substrates in biorefineries, in fermentative processes using yeasts to produce various bioproducts of interest, to make the process more sustainable and meet the sustainable development goals. Therefore, it is necessary to explore biodiversity for yeasts that can provide genes with traits for utilizing these residues or that can be employed in these processes[6].

In this context, understanding the diversity of yeasts in the Araucaria Forest and their potential to grow on carbohydrates that constitute residues can contribute to improve processes and make them more sustainable. Thus, the present work aimed to isolate and evaluate the growth profile of yeasts isolated from the Araucaria Forest, looking forward to using them in the exploitation and treatment of waste.

2 MATERIAL & METHODS

The collecting site chosen was the Chapecó National Forest, which belongs to the Mixed Ombrophilous Forest vegetation type. The collecting zone, with coordinates 27°5'46.00"S 52°47'14.60"W, was the Araucaria Trail, situated in the Guatambú/SC part. Samples of leaf litter (decaying leaves and branches, compound leaves, and Araucaria pine cones), surface soil, and bark from the trunk of *Araucaria angustifolia* were collected and stored in sterile Falcon tubes. For the isolation process, 125 mL Erlenmeyer flasks containing 25 mL YNB medium (6.7 $g \cdot L^{-1}$ yeast nitrogen base without amino acids, pH 5.0) with 10 $g \cdot L^{-1}$ xylose, 0.02 $g \cdot L^{-1}$ chloramphenicol, and 1 g of the collected sample were initially used. The flasks were maintained in a shaker (145 rpm) at 11 °C or 30 °C until high turbidity was observed. The isolation of strains from the collected samples was performed

according to the protocol described by Bazoti et al.[7]. The isolated strains were stored in an ultra-freezer at -80 °C with 30% glycerol.

For micro-scale cultivation, cells were pre-cultivated in 50 mL Erlenmeyer flasks containing 10 mL of YP medium (20 g·L^{-1} peptone, 10 g·L^{-1} yeast extract) with 20 g·L^{-1} glucose for 48 hours at 30 °C with shaking at 145 rpm. Subsequently, 5 µL of the pre-culture were inoculated into wells of a 96-well microplate containing 100 µL of YNB medium with 20 g·L^{-1} carbohydrate. The carbohydrates used in the micro-scale cultivation medium were glucose, xylose, cellobiose, maltose, fructose, arabinose, galactose, rhamnose, and fructose to evaluate the carbohydrate consumption profile of each isolate. The plate was then sealed and incubated in a Tecan GENios microplate reader at 30 °C with 160 linear shakes (1 mm amplitude) per minute. Cell growth was assessed by monitoring optical density (OD) readings every 15 minutes at a wavelength of 600 nm [8].

3 RESULTS & DISCUSSION

After cultivating the collected samples and performing several serial streakings until obtaining pure cultures at temperatures of 11 °C and 30 °C, a total of 32 isolates were obtained from the samples (Table 1). Of these, 8 were from soil samples, 8 from Araucaria bark, and 16 from leaf litter samples, being designated with the prefix CHAP, following the numbering of the isolate collection of the Laboratory of Yeast Biochemistry (LabBioLev). For the same soil samples used at both temperatures, a higher number of isolates was observed at 11 °C, and the same was noted for the leaf litter samples. However, for the Araucaria bark samples, there was no difference in the number of isolates at both temperatures. Due to the soil temperature varying with depth and solar incidence on the surface, yeasts that are adapted to the minimum average winter day temperatures of 11 °C in the municipality of Guatambú-SC tend to tolerate the stress conditions imposed by the environment.

Table1 Nomenclature, cultivation temperature and sample origin of the strains

"CHAP" Strain	Sample	Cultivation temperature	"CHAP" Strain	Sample	Cultivation temperature
254	Soil	11 °C	272	Leaf litter	11 °C
255			273		
257			274		
258			275		
259			276	Soil	30°C
260	Bark		277		
261			278		
262			279	Bark	
263			280		
264	Leaf litter		281		
265			282		
266			283	Leaf litter	
267			284		
268			285		
270			286		
271			287		

In order to evaluate the profile of the isolates regarding their affinity to carbohydrates that constitute residues which can be utilized in fermentative processes, 16 out of the 32 isolates have been tested so far in micro-scale cultures for 72 hours in eight different carbohydrates. After this cultivation, with the data obtained, the maximum specific growth rate [µmax (h^{-1})] of the strains was estimated, as shown in Figure 1.

All isolates were able to grow on most of the carbohydrates tested (Figure 1). Additionally, it is possible to observe the similarity in phenotypic profiles among some isolates, such as those between the strains CHAP-265 and CHAP-267, and between CHAP-271 and CHAP-272, which did not grow in media containing arabinose, galactose, and rhamnose. On the other hand, CHAP-265 and CHAP-267 showed high µmax values in carbohydrates like xylose and cellobiose, these were respectively for xylose of 0.427 ± 0.057 h^{-1} and 0.300 ± 0.059 h^{-1}, and for cellobiose of 0.449 ± 0.098 h^{-1} and 0.428 ± 0.010 h^{-1}, as both were isolated from the same source, leaf litter, but from different collection points.

Similarly, the isolated strains showed low preference for arabinose and rhamnose (Figure 1), except for the strain CHAP-261, which had a µmax of 0.385 ± 0.053 h^{-1}, indicating potential for utilizing lignocellulosic wastes in the production of compounds like arabitol, which can be used as a sweetener, or even ethanol[9,10]. Another noteworthy point is that, since the isolation process was designed to find potential xylose-fermenting or xylitol-producing yeasts, four of the 15 analyzed strains showed high µmax values on xylose, with strain CHAP-281 standing out with the highest value (0.444 ± 0.036 h^{-1}).

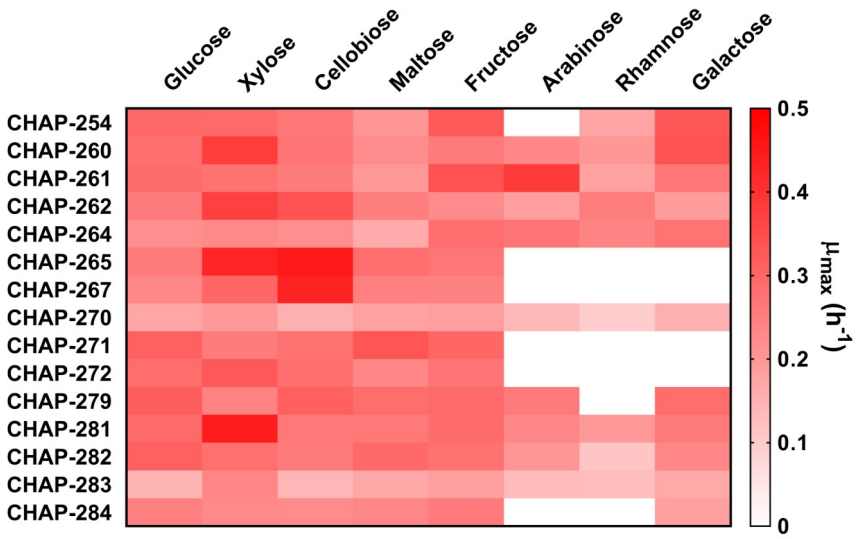

Figure 1 Maximum growth rate of the strains cultivated on a microscale. Each value presented represents the average of two individual cultures. The color scale indicates how fast the strain grew on the respective carbohydrate

4 CONCLUSION

The isolated strains were shown to be capable of growing on sugars present in lignocellulosic residues that can be utilized in fermentation processes. The analysis of microscale cultivation revealed the similarity in the carbohydrate phenotypic profile of the isolates. The strain CHAP-261 exhibited a high maximum specific growth rate in a medium containing arabinose, warranting further investigation into its metabolism in the presence of this carbohydrate. Notably, strains CHAP-265 and CHAP-281 demonstrated the highest μmax values on xylose among the isolates. In conclusion, this study highlights the importance of understanding biodiversity when searching for new yeasts for residues-based bioprocesses.

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ACKNOWLEDGEMENTS

This work is part of the National Institute of Science and Technology (INCT) “Yeasts: Biodiversity, preservation, and biotechnological innovation”. It is supported by grants and fellowships from the Brazilian National Council for Scientific and Technological Development (CNPq, grant numbers 406564/2022-1, 150719/2023-0, and 308830/2023-7), the Research and Innovation Funding Agency of the State of Santa Catarina (FAPESC, grant number 2023TR000234), and the Research Promotion Program from the Federal University of Fronteira Sul (UFFS, grant numbers PES-2022-0221, PES-2023-0349, PES-2023-0351, and PES-2023-0352).