PROSPECTING OLEAGINOUS AND XYLOSE-ASSIMILATING YEASTS IN ARAUCARIA FORESTS

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ABSTRACT

The use of oleaginous yeasts to produce biofuels replaces vegetable oils that require time and cultivation space, thus increasing the efficiency of this production. By combining the specialty of oleaginous yeasts to produce lipids with the consumption of lignocellulosic raw material, rich in sugars such as glucose and xylose, the reuse of waste in industries is increased and consequently, the reduction of unnecessary disposal and loss of these materials to landfills. For this reason, this work sought to prospect for oleaginous and xylose-assimilating yeasts in samples of soil, litter, and tree bark from Araucaria Forests. Cell growth on glucose was also evaluated. Yeasts were tested at 30°C and 11°C. Among 28 strains analyzed, 16 were capable of accumulating lipids to different degrees. Furthermore, all yeasts were able to grow on the two carbon sources tested and at two different temperatures. While the strains CHAP-271, CHAP-272, and CHAP-273 showed the highest maximum OD results at 30°C, the strains CHAP-300, CHAP-301, CHAP-302, and CHAP-303 stood out at 11°C. Our data, therefore, indicate the potential of yeasts from Araucaria Forests to produce lipids from lignocellulosic waste.

Keywords: Cellular growth. Low temperature. Lipid. *Yarrowia lipolytica*.

1 INTRODUCTION

The increase in waste production and greenhouse gas emissions indicate the need for behavioral changes, which depend on replacing fossil fuels with biofuels and on the search for new raw materials. Biodiesel, for example, is manufactured from vegetable oils that could be replaced by fatty acids from oleaginous yeast, which uses a smaller cultivation area and has greater production efficiency[1]. Furthermore, oleaginous yeasts are also capable of using industrial waste as a carbon source, reducing production costs[2]. This strategy, however, needs to be optimized to become more economically attractive, hence the importance of prospecting for new yeasts.

Yeasts are considered oleaginous when they have the capacity to accumulate amounts of lipids greater than or equal to 20% of their cellular dry weight. This storage occurs when some carbon source is available in excess and another nutrient, such as nitrogen, is limited[2,3]. Therefore, the present work aimed to identify oleaginous yeasts isolated from soil, litter, and tree bark from Araucaria Forests. In parallel, to verify the potential use of these microorganisms in biorefineries whose feedstocks are plant residues, their growth was evaluated in media with glucose and xylose at two different temperatures.

2 MATERIAL & METHODS

The yeasts were isolated from samples collected in Araucarias Forests of three national conservation units located in the state of Santa Catarina: Chapecó National Forest, Araucarias National Park, and São Joaquim National Park. Samples of soil, litter, and bark from Araucaria trunks were collected. For yeast isolation, the samples were inoculated in Erlenmeyer flasks with YNB liquid medium (6.7 g/L of nitrogen base) with 10 g/L of xylose and 0.2 g/L of chloramphenicol. The flasks were kept under shaking at 145 rpm alternately at 30°C or 11°C. After 2, 4, and 6 days of cultivation, loops of 10 µL of medium were streaked in Petri dishes with solid YNB medium containing 10 g/L xylose and 20 g/L agar. The plates were also incubated at 30°C or 11°C. After growth, colonies with typical yeast morphology were isolated and stored at -80°C[4]. Among all the strains isolated, 28 were used in this study. For all assays carried out in this study, yeasts were pre-cultured in YPD solid medium (10 g/L yeast extract and 20 /L peptone, 20 g/L glucose, and 20 g/L agar) for 48 h at 30°C.

For the screening of oleaginous yeasts, 1 µL of pre-cultured cells was spiked into Petri dishes with solid medium containing 23 g/L glucose, 0.3 g/L peptone, 0.5 g/L yeast extract, 7 g/L monopotassium phosphate, 1.06 g/L anhydrous dibasic sodium phosphate, 3.07 g/L magnesium sulfate heptahydrate, 20 g/L of agar, added with 100x diluted Rhodamine B stock solution (in 1 g/L ethanol). Plates were then incubated for 48 h at 30°C. In this assay, oleaginous yeast colonies were expected to turn pink, and non-oleaginous yeast colonies to turn white[1]. The yeasts *Saccharomyces cerevisiae* PE-2 and *Yarrowia lipolytica* UFMG-CMY6114 were used as negative and positive controls, respectively. The yeast classification was carried out using the ImageJ software, which measures the gray level intensity of the pixels in a photo of the yeast colony[5]. For this assay, we selected the average intensity values. The yeasts were classified using a scale in which the value "zero" is equivalent to the lowest average intensity measured (given by the pink color of the oleaginous yeasts) and the value "one" is equivalent to the average intensity of the negative control (given by the white color of non-oleaginous yeasts). Thus, the lower the average intensity, the pinker the

colony. The positive control was used to ensure that the screening was valid. The values of each strain were then calculated using Equation 1.

$$YA = Y1 + (Y2 - Y1) \cdot \frac{(XA - X1)}{(X2 - X1)} \quad (1)$$

In which:
YA = Equivalent value on the scale;
Y1 = 0;
Y2 = 1;
XA = Average intensity;
X1 = Negative control's average intensity;
X2 = lowest average intensity measured.

Therefore, scale values between 0 and 0.25 were classified as very oleaginous; between 0.25 and 0.50, as intermediately oleaginous; between 0.50 and 0.75, as little oleaginous; and values between 0.75 and 1 were classified as non-oleaginous.

To evaluate cell growth on a microscale, 1 µL of pre-cultured cells was initially suspended in 1 mL of liquid YNB medium with 20 g/L glucose, and 1 µL of this suspension was inoculated into 100 µL of liquid YNB with 20 g/L glucose or 20 g/L xylose per well in 96-well microplates. The plates were sealed and kept at 30ºC under shaking at 200 rpm for 96 h. The optical density of each well of the plate was measured twice daily at 595 nm. The tests were carried out in triplicate and the standard deviations were always less than 10% of the average value.

3 RESULTS & DISCUSSION

Among the 28 yeasts analyzed, 16 accumulated lipids intracellularly to a greater or lesser extent (Table 1). Four of them (CHAP-291, CHAP-292, CHAP-302, and CHAP-303) accumulated lipids in amounts similar to the control strain UFMG-CMY6114, which to the species *Y. lipolytica*, a yeast widely recognized for its high oleogenic potential[1].

Table 1 Identification and classification of oleaginous yeasts.

Strains	Average Intensity	Scale	Classification
UFMG-CMY6114	122.309	-	Positive control
PE-2	149.133	1	Negative control
CHAP 271	137.605	0.488	Intermediately oleaginous
CHAP 272	133.408	0.302	Intermediately oleaginous
CHAP 273	135.506	0.395	Intermediately oleaginous
CHAP 275	159.554	1	Non-oleaginous
CHAP 276	160.633	1	Non-oleaginous
CHAP 277	160.129	1	Non-oleaginous
CHAP 278	156.245	1	Non-oleaginous
CHAP 279	158.558	1	Non-oleaginous
CHAP 280	154.193	1	Non-oleaginous
CHAP 281	154.265	1	Non-oleaginous
CHAP 282	150.836	1	Non-oleaginous
CHAP 283	145.579	0.842	Non-oleaginous
CHAP 284	141.125	0.645	Little oleaginous
CHAP 285	154.290	1	Non-oleaginous
CHAP 286	156.816	1	Non-oleaginous
CHAP 287	148.970	0.993	Little oleaginous
CHAP 291	131.150	0.202	Very oleaginous
CHAP 292	128.996	0.106	Very oleaginous
CHAP 293	138.681	0.536	Little oleaginous
CHAP 300	147.267	0.917	Non-oleaginous
CHAP 301	139.422	0.569	Little oleaginous
CHAP 302	126.598	0	Very oleaginous
CHAP 303	130.090	0.155	Very oleaginous
CHAP 387	151.415	1	Non-oleaginous
CHAP 390	137.484	0.483	Intermediately oleaginous
CHAP 391	132.252	0.251	Intermediately oleaginous
CHAP 395	151.776	1	Non-oleaginous
CHAP 397	136.502	0.439	Intermediately oleaginous

All strains were able to grow using glucose and xylose as carbon sources. As expected, cell growths at 30°C (Figure 1A) were greater than those at 11°C (Figure 1B). Even so, the production of cellular biomass at 11°C deserves to be highlighted, given the effectiveness of the growth observed at a temperature that is not favorable for several species of microorganisms. Interestingly, two of the yeasts that stood out most at 11°C, CHAP-302 and CHAP-303, are also among those that most accumulated lipids intracellularly (Table 1). Both strains, therefore, present desirable phenotypes for bioprocesses that could produce lipids at lower temperatures, which are known to improve lipid production by yeast cells[6].

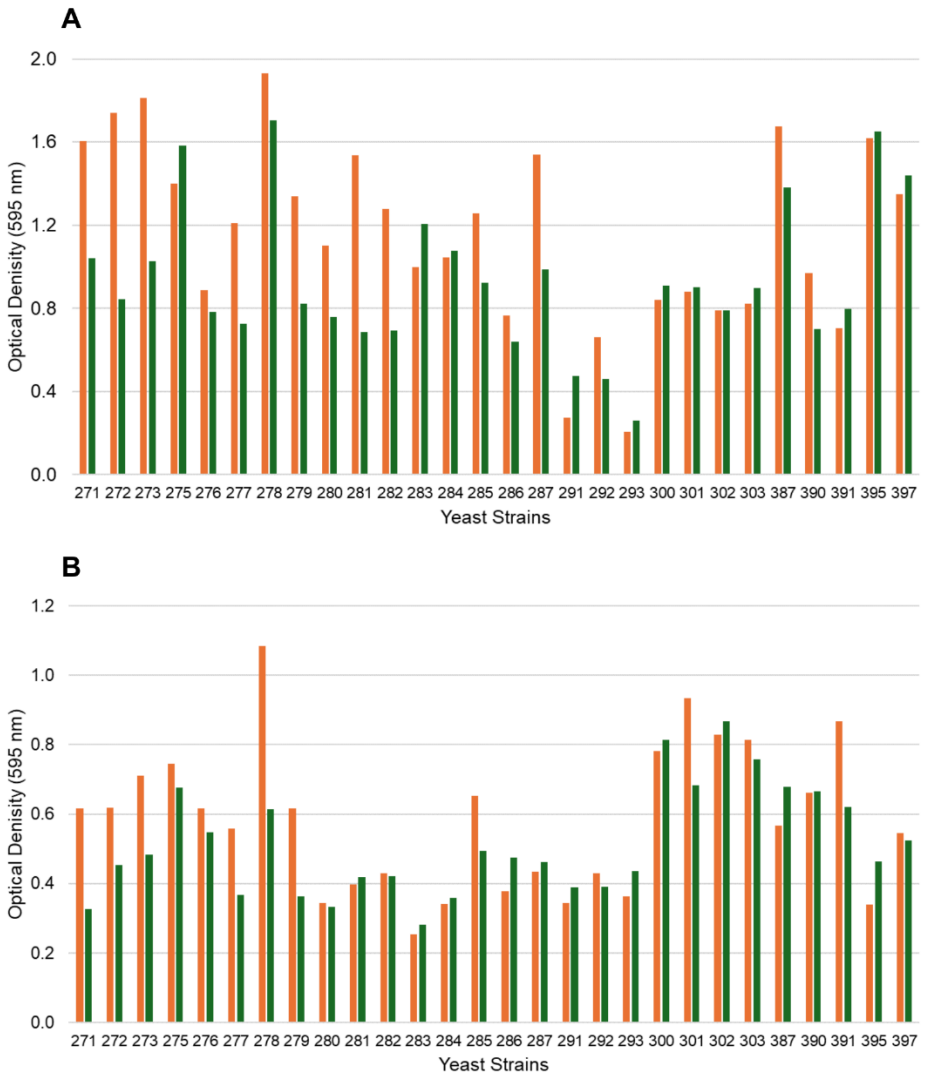

Figure 1 Maximum optical density achieved by yeasts at 30°C (A) or 11°C (B) on microscale cultures with YNB media containing glucose (orange bars) or xylose (green bars) as carbon sources.

4 CONCLUSION

The results demonstrate the biotechnological potential of yeasts from soil, litter, and tree bark from Araucarias Forests. The strains analyzed and classified as oleaginous were capable of consuming glucose and xylose, both at 30°C and 11°C. Oleaginous yeasts with these characteristics have interesting qualities that add value to the biofuels industry, as their oils can replace vegetable oils in biodiesel production.

REFERENCES

[1] Lei, Y., Wang, X., Sun, S., He, B., Sun, W., Wang, K., Chen, Z., Guo, Z., & Li, Z. (2024). Science of The Total Environment, 919, 170385. https://doi.org/10.1016/j.scitotenv.2024.170385.
[2] Niehus, X., Casas-Godoy, L., Vargas-Sánchez, M., & Sandoval, G. (2018). Journal of Lipids, 2018, 1–8. https://doi.org/10.1155/2018/5325804.
[3] Mierke, F., Brink, D. P., Norbeck, J., Siewers, V., & Andlid, T. (2023). Fungal Genetics and Biology, 166, 103783. https://doi.org/10.1016/j.fgb.2023.103783.
[4] Tadioto, V., Milani, L. M., Barrilli, É. T., Baptista, C. W., Bohn, L., Dresch, A., Harakava, R., Fogolari, O., Mibielli, G. M., Bender, J. P., Treichel, H., Stambuk, B. U., Müller, C., & Alves, S. L. (2022). World Journal of Microbiology and Biotechnology, 38(2), 35. https://doi.org/10.1007/s11274-021-03221-0.
[5] Hannickel, A., Silva M. H. P., Barros H. L., & Albuquerque M. P. (2012). Revista Militar de Ciência e Tecnologia, 19(4). 16-26.
[6] Da Silva, T. L., Fontes, A., Reis, A., Silva, C., & Gírio, F. (2023). Fermentation, 9(12), 1013. https://doi.org/10.3390/fermentation9121013.

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 Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES), 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-2023-0350, PES-2023-0351, and PES-2023-0352).