PROSPECTING WILD YEASTS FOR XYLOSE DEGRADATION POTENTIAL

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ABSTRACT

The research in biotechnology explores new microbial sources for industrial applications, from food production to waste conversion into bioenergy. The search for promising microorganisms in natural environments drives these advancements, linked to the development of new processes and bioengineering. In this context, the aim of this study is to bioprospect wild yeast strains capable of xylose consumption for industrial applications, such as the production of ethanol and organic acids from lignocellulosic biomass. The strains were isolated and purified from various environments, including sugarcane bagasse, orchids, tree trunks, and soil, using serial dilution and plating techniques. The yeasts were subjected to a fermentation process to evaluate their potential for biomass growth and xylose consumption. Results were assessed by determining dry weight and reducing sugars using the 3,5-dinitrosalicylic acid (DNS) method. Forty-two yeasts were isolated, and it was found that 19% of the strains exhibited good performance in xylose consumption, showing potential for future applications in environmental biotechnology and bioenergy.

Keywords: Bioprocess. Biotechnology. Isolation. Lignocellulosic biomass. Sugar metabolism.

1 INTRODUCTION

Microorganism isolation is a key microbiological procedure aimed at obtaining strains with significant biotechnological and industrial potential, often involving the discovery of new strains or genes that can lead to the development of new products or bioprocesses[1].

The utilization of lignocellulosic residues from biofuel production has emerged as a promising source for second-generation biofuels, particularly from agricultural residues, such as bagasse and straw generated in first-generation processes. One challenge in this context is fermenting cultivation media derived from the pretreatment and hydrolysis of residues, which is rich in various hexoses and pentoses that are only partially utilized, if at all, by *Saccharomyces cerevisiae*, the yeast commonly used in industrial processes[2]. Therefore, the search for microorganisms capable of metabolizing both hexoses and pentoses present in cellulose and hemicellulose is crucial for advancing second-generation biofuel processes, thereby increasing the yield and profitability of these processes[3,4,5].

Yeasts are among the most interesting microorganisms for industrial processes due to their versatility and unique metabolic capacities, prompting increased interest in isolating new strains[6]. These microorganisms inhabit diverse ecological niches and can utilize a wide range of substrates, hence their isolation from varied environments[7]. Recently, thermotolerant yeasts capable of withstanding temperatures up to 45°C were isolated from bovine rumen, targeting application in simultaneous saccharification and fermentation (SSF) processes for bioethanol production[8]. For instance, Poomani et al.[9] isolated *Pichia kudriavzevii* from bovine rumen for xylanase production and ethanol via SSF. Other environments such as decomposing wood[5], fruits[4] and food[4] also hold potential microbiomes for isolating microorganisms relevant to biotechnology.

In this context, there has been an increase in studies on yeast isolation, particularly unicellular microorganisms from the Kingdom Fungi capable of converting pentoses, mainly xylose, into ethanol. This effort aims to develop co-culture processes with *Saccharomyces cerevisiae*[10] or through genetic engineering to isolate key genes[11], or even using them as the sole fermenting organism[12]. The objective of this study is to prospect natural environmental (sugarcane bagasse, orchids, wood, and soil) and evaluate the potential of yeasts for xylose consumption, aiming at application in bioprocesses.

2 MATERIAL & METHODS

Yeast strains used in this work were isolated from samples of soil, decaying wood, sugarcane bagasse, and orchids. Five grams of each sample were transferred to Erlenmeyer flasks (150 mL) containing 50 mL of 0.85% NaCl and incubated with shaking at 150 rpm for 30 minutes at 30°C[13].

After this, 1 mL aliquots were used to prepare three serial dilutions of the sample (1:10, 1:100, and 1:1000) using 0.85% NaCl as a diluent. 0.1 mL of each dilution was transferred to Petri dishes containing solid YPX medium (1% yeast extract, 2% peptone, 2% xylose) with 100 µg/mL chloramphenicol via Drigalski spatula plating. Each plating was performed in triplicate, and plates were incubated at 28°C for 48 hours.

After colony growth, each strain underwent a purification process where the total number of colonies on each plate was counted, and the square root of the value was applied. The result was considered the number of colonies that would be randomly re-inoculated on Petri dishes containing solid YPX medium for 48 hours at 28°C. After growth, one randomly chosen colony from each plate was re-streaked twice. Following this procedure, the isolated strain was considered purified.

Morphological analysis was conducted to confirm they were yeasts through microscopic observation using lenses from 40x to 100x. Characteristics such as shape (round and elongated) and reproduction mode (budding and fission) were observed. Strains were stored in penicillin vials containing solid YPX medium in slants and 20% glycerol at 4°C until use.

To select yeasts capable of fermenting xylose, experiments were conducted using the isolates. Pre-culture was performed on solid YPX (with agar) at 30°C for 48 hours. After growth, cells were transferred to 7 mL of liquid YPX medium for 24 hours at 30°C in an orbital shaker at 140 rpm.

For fermentations, 1 mL of the yeast cell culture was transferred to 9 mL of YPX medium containing 15 g/L xylose, 3 g/L yeast extract, 3.5 g/L peptone, and supplemented with 2 g/L KH_2PO_4, 1 g/L $MgSO_4 \cdot 7H_2O$, and 1 g/L $(NH_4)_2SO_4$[5]. Samples were conducted in triplicate and maintained in an orbital shaker at 90 rpm at 30°C for 96 hours.

Total reducing sugars (TRS) were quantified using the dinitrosalicylic acid (DNS) method[14]. The method was conducted in a 96-well plate. A 62.5 µL aliquot of appropriately diluted sample was mixed with DNS reagent. The reaction blank was conducted with distilled water. Samples were incubated for 10 minutes at 100°C. After the reaction was stopped in an ice bath, 875 µL of distilled water was added to the samples, which were homogenized. 200 µL were pipetted into the wells, and the reading was conducted at 540 nm. For quantification, a standard curve was constructed with xylose (Sigma Aldrich) in concentrations of 0.10 to 2.5 g/L, with an R^2 of 0.9967.

3 RESULTS & DISCUSSION

Yeasts are unicellular, eukaryotic microorganisms belonging to the Kingdom Fungi. These cells, which are spherical or oval, primarily reproduce through budding or fission (Fig. 1). They are chemoheterotrophic absorbers, meaning they metabolize organic compounds to obtain energy and nutrients[7]. In nature, yeasts colonize a variety of sugar-rich environments, such as flowers, leaves, and fruits, and are also found in soil, water, food, and wood.

In this study, four natural environments were explored for the isolation of xylose-consuming yeasts: soil, sugarcane bagasse, orchids, and decaying wood. In total, 42 yeasts were isolated, with 33.33% from orchids, 30.95% from decaying wood, 16.67% from soil, and 19.05% from sugarcane bagasse. After the isolation and purification process, morphological differences between the yeasts were analyzed, as noted in the images below.

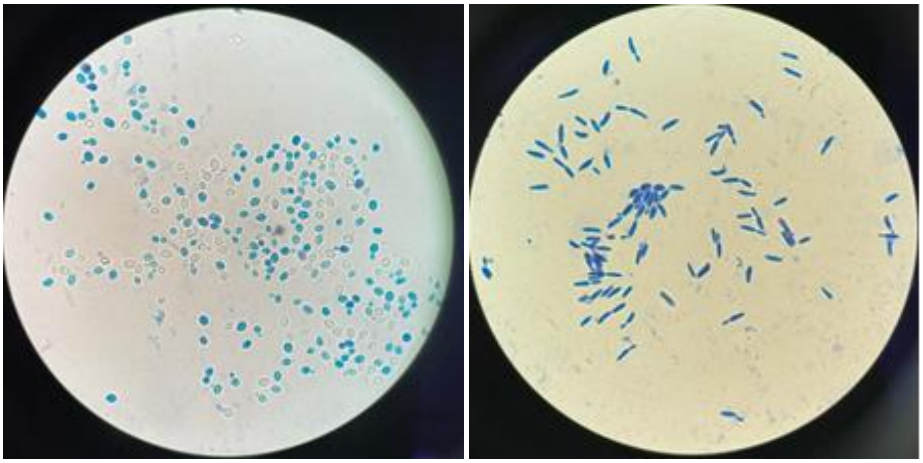

Figure 1 Sample Ba3 (round and budding) and T34 (elongated and fission) (100x).

Over a 96-hour period, the fermentation capacity of cultured yeast was assessed through the measurement of xylose consumption. The 42 isolated yeasts presented a range of 4% to 47% of xylose consumption. Table 1 contains the 8 strains that consumed a significant percentage of xylose. Thus, it is evident that different yeasts isolated from different environments and with different morphologies can present biotechnological potential for application in industrial fermentative media, such as lignocellulosic hydrolysates. All the isolated strains obtained were cataloged and cryopreserved to be part of the strain collection at the Laboratory of Bioprocess Engineering and Biotechnology at the Federal University of Paraná (Curitiba, PR).

Table 1 Data of isolated yeasts that demonstrated the capacity of xylose consumption in 96-hour fermentation. Experiments were conducted in triplicate, and data are presented as mean ± standard deviation.

Yeast code	Isolation Source	Biomass (g/L)	Final xylose concentration (g/L)	Xylose consumption (%)
BA1	Sugarcane bagasse	2.19 ± 0.25	16.02 ± 1.41	18.51
BA3	Sugarcane bagasse	2.60 ± 0.10	13.78 ± 0.77	26.37
OR32	Orchid	0.60 ± 0.13	15.27 ± 0.38	19.72
SO11	Soil	1.78 ± 0.33	9.82 ± 0.43	46.81
SO14	Soil	1.36 ± 0.10	16.87 ± 2.05	20.45
SO16	Soil	1.15 ± 0.38	11.38 ± 0.39	31.28
TR1	Decaying wood	0.73 ± 0.09	18.88 ± 1.35	14.03
T39	Decaying wood	0.34 ± 0.11	14.66 ± 0.33	21.08

Among the tested yeast isolates, the most proficient in xylose consumption was SO11, isolated from soil, with a consumption of approximately 47%. The microbiological diversity allows a range of results to be observed in the literature. For instance, Martins et al.[15] isolated the strain *Candida tropicalis* S4 from a grape leaf and achieved 100% xylose consumption. Another study conducted by Costa et al.[16] demonstrated a xylose consumption of 55% by the yeast *Diutina rugosa* isolated from bovine rumen. These results highlight the variability in xylose consumption, underscoring their potential for industrial applications.

Considering this scenario, there is a prospect for identifying this SO11 yeast. Among the xylose-consuming species, examples include *Wickerhamomyces*[5], *Torulaspora*[16], *Scheffersomyces*[17], *Pichia*[4], *Spathaspora*[18], *Diutina*[16], *Candida*[17], *Schwanniomyces*[17], *Sugiwamaella*[16,17] and others.

4 CONCLUSION

This study isolated 42 yeast strains from four environments (soil, sugarcane bagasse, orchids, and decaying wood) and they exhibited different morphological characteristics, ranging from colony growth aspects to cell growth morphology. Among these microorganisms, 8 showed potential for xylose consumption in YPX medium over 96 hours of fermentation, with a notable performance from a yeast strain isolated from soil (SO11), which exhibited 47% xylose consumption. Prospects of this study include the identification and application in more complex media, such as sugarcane lignocellulosic hydrolysate.

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