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SCREENING OF ENVIRONMENTAL YEASTS FROM THE CERRADO FOR THE PRODUCTION OF HYDROLYTIC ENZYMES OF BIOTECHNOLOGICAL INTEREST

TRIAGEM DE LEVEDURAS AMBIENTAIS DO CERRADO QUANTO À PRODUÇÃO DE ENZIMAS HIDROLÍTICAS DE INTERESSE BIOTECNOLÓGICO

CRIBADO DE LEVADURAS AMBIENTALES DEL CERRADO PARA LA PRODUCCIÓN DE ENZIMAS HIDROLÍTICAS DE INTERÉS BIOTECNOLÓGICO

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ABSTRACT

Environmental yeasts represent an underexplored source of novel hydrolytic enzymes with biotechnological potential. The objective of this study was to evaluate the potential for hydrolytic enzyme production by environmental yeasts from the Cerrado. The tested yeasts (18 isolates) were derived from lobeira (*Solanum lycocarpum*) fruits, cashew fruits and pseudofruits (*Anacardium occidentale*), pequi (*Caryocar coriaceum*) fruits, the body surface of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), and corn kernels (*Zea mays*). The isolates were grown and/or reactivated on Potato Dextrose Agar (PDA) medium at 25 ± 3 °C for 48 h. Screening for the production of amylases, cellulases, pectinases, and proteases on solid medium was performed, and enzyme activity indices (EAI) were calculated. Of the total number of isolates tested (18), 44.4% demonstrated cellulolytic activity, 27.8% amylolytic activity, and 22.2% pectinolytic activity. None demonstrated proteolytic activity. Isolates LC2, LC1, and P2L1 demonstrated activity for three of the four enzymes tested (cellulase, amylase, and pectinase). Isolate LC2 exhibited the highest EAI values, achieving the best EAI for cellulolytic activity under acidic pH conditions. It was concluded that yeasts with biotechnological potential, such as isolates LC2, LC1, and P2L1, can be obtained from

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environmental substrates, especially edible fruits and pseudofruits. Studies such as this reinforce the importance of investing in the conservation and enhancement of Cerrado biodiversity.

Keywords: Biotechnology. Biodiversity. Enzymes. Yeasts.

RESUMO

Leveduras ambientais representam uma fonte pouco explorada de novas enzimas hidrolíticas com potencial biotecnológico. O objetivo deste trabalho foi avaliar o potencial de produção de enzimas hidrolíticas por leveduras ambientais do Cerrado. As leveduras testadas (18 isolados) eram provenientes de frutos da lobeira (*Solanum licocarpum*), frutos e pseudofrutos de caju (*Anacardium occidentale*), fruto de pequi (*Caryocar coriaceum*), superfície corpórea de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) e de grãos de milho (*Zea mays*). Os isolados foram cultivados e/ou reativados em meio Batata Dextrose Ágar (BDA), a 25 ± 3°C, 48 h. Foi realizada triagem para produção de amilases, celulases, pectinases e proteases em meio sólido e foram calculados índices de atividade enzimática (IAE). Do total de isolados testados (18), 44,4% demonstraram atividade celulolítica, 27,8% amiloítica e 22,2% pectinolítica. Nenhum apresentou atividade proteolítica. Os isolados LC2, LC1 e P2L1 apresentaram atividade para três (celulase, amilase e pectinase) das quatro enzimas testadas. O isolado LC2 apresentou os melhores IAEs, atingindo melhor IEA para atividade celulítica em pH ácido. Concluiu-se que leveduras com potencial biotecnológico, como os isolados LC2, LC1 e P2L1, podem ser obtidas a partir de substratos ambientais, sobretudo frutos e pseudofrutos comestíveis. Estudos como este reforçam a importância de se investir em conservação e valorização da biodiversidade do Cerrado.

Palavras-chave: Biotecnologia. Biodiversidade. Enzimas. Leveduras.

RESUMEN

Las levaduras ambientales representan una fuente poco explorada de nuevas enzimas hidrolíticas con potencial biotecnológico. El objetivo de este estudio fue evaluar el potencial de producción de enzimas hidrolíticas por levaduras ambientales del Cerrado. Las levaduras evaluadas (18 aislados) se obtuvieron de frutos de lobeira (*Solanum lycocarpum*), frutos y pseudofrutos de anacardo (*Anacardium occidentale*), frutos de pequi (*Caryocar coriaceum*), de la superficie corporal de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) y de granos de maíz (*Zea mays*). Los aislados fueron cultivados y/o reactivados en medio Agar Papa Dextrosa (PDA) a 25 ± 3 °C durante 48 h. Se realizó un cribado para la producción de amilasas, celulasas, pectinasas y proteasas en medio sólido, y se calcularon los índices de actividad enzimática (IAE). Del total de aislados evaluados (18), el 44,4 % presentó actividad celulolítica, el 27,8 % actividad amilolítica y el 22,2 % actividad pectinolítica. Ninguno presentó actividad proteolítica. Los aislados LC2, LC1 y P2L1 demostraron actividad para tres de las cuatro enzimas evaluadas (celulasa, amilasa y pectinasa). El aislado LC2 presentó los valores más altos de IAE, alcanzando el mejor índice de actividad celulolítica en condiciones de pH ácido. Se concluyó que levaduras con potencial biotecnológico, como los aislados LC2, LC1 y P2L1, pueden obtenerse a partir de sustratos ambientales, especialmente de frutos y pseudofrutos comestibles. Estudios como este refuerzan la importancia de invertir en la conservación y valorización de la biodiversidad del Cerrado.

Palabras clave: Biotecnología. Biodiversidad. Enzimas. Levaduras.

1 INTRODUCTION

Yeasts are unicellular microorganisms, divided into two phyla: Ascomycota and Basidiomycota, with diameters ranging from 1 to 5 µm (MOREIRA et al., 2006). They are eukaryotic and heterotrophic cells, have a rigid cell wall, and reproduce sexually or asexually (PEIXOTO, 2006). Several yeast species have been detected in association with plant species, gaining notoriety due to their ability to perform functions relevant to plant health and/or to exhibit biotechnological potential (BONFIM, 2014; MACHADO, 2015).

Yeasts have also been identified living in symbiotic associations with various terrestrial or aquatic insects (MELO et al., 2019; SANTOS et al., 2019), including termites, bees, caterpillars, and ants. Typically, these microorganisms are found on the host's external cuticle or in the intestine, providing protection against pathogens, parasitoids, and other organisms through the synthesis of specific toxins or by modulating the host's immune system (MELO et al., 2019).

Yeast and filamentous fungi can produce a wide variety of enzymes, which are complex proteins specialized in catalyzing biological reactions that occur within animal and plant cells and microorganisms, facilitating and accelerating most of the biochemical reactions (TAVAFI et al., 2017, TEIXEIRA et al., 2022). Because they possess an active site, enzymes exhibit specificity both for their substrates and for the type of reaction they catalyze. Due to these characteristics, enzymes are catalysts widely used in various industrial segments and in chemical analysis and diagnostic methods, among others (FARINAS, 2011).

Among the enzymes that can be produced by yeasts and filamentous fungi are hydrolytic enzymes, which are widely used in industrial processes, particularly in the degradation of natural substances. In the textile industry, the most commonly used enzymes are amylases, cellulases, pectinases, and oxidoreductases. In detergent production industries, cellulases, lipases, proteases, and oxidoreductases are almost always used. In the food industry, cellulases, lactases, lipases, pectinases, proteases, and oxidoreductasse are commonly used, while in the paper and leather industries, lipases, oxidoreductases, xylanases, and proteases are primarily used (OLIVEIRA et al., 2006, MOTTA et al., 2023, KUMAR et al., 2023).

Several screening studies for the production of hydrolytic enzymes from yeasts grown in environmental substrates have been conducted, yielding promising results (ZAKY et al., 2014; SOHAIL et al., 2022; SUKMAWATI et al., 2023). However, the main enzyme preparations used by the industry originate from filamentous fungi (MACHADO, 2015; BARBOSA et al., 2020), which highlights a potential field of yeast use that still needs further investigation, exploration and improvement. Therefore, the objective of this study was to

evaluate the potential for production of hydrolytic enzymes (amylase, cellulase, pectinase, and protease) by environmental yeasts from the Cerrado, one of the biodiversity hotspots on planet Earth.

2 METHODOLOGY

2.1 REACTIVATION AND VERIFICATION OF MORPHOLOGICAL CHARACTERIZATION OF ENVIRONMENTAL YEASTS

Yeasts from the microbial culture collection of Laboratory 4 of UFOB/CMLEM (Table 1) were reactivated and their morphological characterization was verified against the respective records before being used in the enzymatic screening tests of this study, as described below. Reactivation occurred on Potato Dextrose Agar (PDA) medium, with incubation at 25 ± 3°C for 48 h.

Table 1

Morphospecies of yeasts used by codes and the source of obtainment

Origin of yeasts	Number of isolates and collection codes
Endophytic from cashew fruits and pseudofruits (*Anacardium occidentale* L.)	02 isolates from fruits: C5L2 e C5L1. 04 isolatres from pseudofruits: P2L1, P3L1, P4L1 e P7L1.
Endophytic from pequi fruit (*Caryocar coriaceum* Wittm)	01 isolate: P1L1.
Body surface of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)	06 isolates: L1L1, L1L2, L1L10, L5L2, L5L3, L10L2.
Corn grains (*Zea mays* L.)	02 isolates: M316 e M217.
Total isolates	15

Source: Authors (2026).

The yeasts obtained from cashew fruits and pseudofruits (*A. occidentale*) and pequi (*C. coriaceum*) were isolated as endophytes in previous studies conducted by researchers from the "Biotechnology Engineering" research group at UFOB, in the Cerrado biome, in western Bahia. The yeasts from the body surface of *S. frugiperda* were isolated, characterized and preserved by Silva et al. (2023), while yeasts isolated from corn grains (*Zea mays*) were isolated by Silva et al. (2024). These yeasts were morphologically characterized and preserved in GYP medium (2% glucose, 1% yeast extract and 0.5% peptone) plus Glycerol (15%) (SPENCER; SPENCER, 1996).

2.2 ISOLATION AND MORPHOLOGICAL ANALYSIS OF YEASTS FROM LOBEIRA (*SOLANUM LYCOCARPUM* ST. HIL)

To obtain yeasts from lobeira (*S. lycocarpum*), the fruits were collected directly from plants in March 2023, at Parque Vida Cerrado, located in the municipality of Luís Eduardo Magalhães-BA, at coordinates 12° 4'39.95"S and 45°40'22.62"W. Leaves and flowers were collected to identify the fruit species, and the fruits were sent for microorganism isolation. All samples were packaged in hermetically sealed bags under hygienic conditions and stored under refrigeration for later analysis. For this study, 2 (two) ripe fruits from two different lobeira plants were used.

In the laboratory, the fruits were washed with distilled water and 2% sodium hypochlorite, remaining immersed in the medium for 15 minutes. Then, with the help of a sterile knife, parts of the inner peel and pulp were scraped and transferred to Petri dishes with PDA medium with antibiotic (enrofloxacin 0.1µg/mL). With the help of a Drigalski spatula, the fruit scrapings were spread homogeneously on the surface of the medium, with each part of the fruit cultivated separately. The plates were incubated for seven days at 28±3 ºC. After this period, colonies that resembled yeasts were isolated and cultured on PDA medium for three days.

After this cultivation time, it was possible to perform phenotypic identification based on macroscopic characteristics, observed using an optical microscope at 1000x, as proposed by Kurtzman et al. (1998). The observed characteristics were elevation, aspect, color, shape, size, surface and type of border. The identified yeasts were purified and cultured on PDA medium for three days for later analysis of their enzymatic potencial.

2.3 EVALUATION OF AMYLOLYTIC ACTIVITY

The ability of yeasts to degrade soluble starch as the sole carbon source was evaluated according to the method proposed by Hankin and Anagnostakis (1975), with some modifications. For this, a culture medium composed of 2.0 g of corn starch, 1.0 g of yeast extract, 1.0 g of peptone and 1.5 g of agar was prepared. The yeasts were inoculated onto Petri dishes. The plates were incubated at 28±3º C for 96 h. The test was performed in duplicate.

After the incubation period, 10 mL of Lugol's solution was added to each plate and maintained for 30 minutes. Around the colonies where there was amylolytic activity occurred, a discolored halo was observed due to the absence of intact starch. With the aid of a caliper, the diameter of the halos was measured in three different directions, both of the grown colony and for the degradation halo. Enzymatic activity was determined according to Agnostakis and

Hankin (1975), in wich, from the relationship between the mean diameter of the colony, it was possible to determine the enzymatic activity, expressed as Enzymatic Activity Index (EAI), calculated using the equation below. The results were expressed as the mean of the values obtained, since the tests were performed in duplicate.

2.4 EVALUATION OF CELLULOLYTIC ACTIVITY

The ability of the studied yeasts to degrade cellulose polymers was tested according to the methodology proposed by Strauss et al. (2001) and Buzzini and Martini (2002), in duplicate, with some modifications. The reactivated yeasts and the purified yeasts were inoculated with Petri dishes containing YP-CMC medium (5.0 g of yeast extract, 10.0 g of peptone, 2.0 g of CMC, 10.0 g of agar and 500.0 mL of distilled water) and incubated at 25 ± 3°C for 120 hours. The hydrolysis halos were revealed according to Maijala, et al., (1991), for this purpose, the plates were covered with 10 mL of an aqueous solution of 0.3 g/L Congo red for 30 min and and subsequently discolored with 5 mL of 1.0 M NaCl solution for 15 min. The identification of strains producing cellulolytic enzymes was performed by the presence of a translucent halo around the colony, in contrast with the more intense red coloration of the rest of the medium.

Cellulolytic activity was also evaluated through pH variation, considering that enzymatic reactions are affected by changes in this parameter, wich directly influence the rate of catalyzed reactions (BORZANI et al., 2001). For this, three media with the same composition as the medium described above were prepared. To one medium, an acidic solution (0.01 mol/L HCl) was added, adjusting the pH to 4.0; to another, a basic solution (0.01 mol/L NaOH) was added, adjusting the pH to 8.4; and to the third, no solution was added, maintaining a neutral pH of 6.23.

The determination of enzymatic activity was performed according to the methodology proposed by Agnostakis and Hankin (1975), through the relationship between the mean diameter of the degradation halo and the mean diameter of the colony, expressed as EAI, determined by means of Equation 01. The tests were performed in duplicate and the results were expressed as the mean of the values obtained.

2.5 EVALUATION OF PECTINOLYTIC ACTIVITY

The production of pectinolytic enzymes was tested according to Cattelan (1999) and revealed according to Raju et al., (2013), with some adaptations. The isolates were cultured in solid culture medium composed of 0.18 g of yeast extract, 0.075 g of NaCl, 0.03 g of sucrose, 2.25 g of agar, 0.09 g of Na2HPO4, 0.45 g of KH2PO4, 0.6 g of Pectin, 150 mL of

distilled water, 0.15 mL of NH4Cl solution, 1.5 mL of CaCl2.2H2O solution at 0.01 mol and 0.15 mL of MgSO4.7H2O at 1 mol. The medium and solutions were autoclaved separately at 100ºC for 15 min. After autoclaving, the solutions were mixed with the medium and subsequently poured into Petri dishes, onto which the yeasts were inoculated and incubated at 28 ± 3°C for 72 h, in duplicate.

After the incubation period, the Petri dishes were covered with Lugol's solution, for 15 minutes. Lugol's solution reacts with pectin, forming a brownish-colored complex. Isolates that showed a clear yellowish halo around the colonies were considered pectinase producers.

2.6 EVALUATION OF PROTEOLYTIC ACTIVITY

The ability of the studied yeasts to exhibit proteolytic activity was evaluated using the methodology proposed by Ribeiro Júnior (2015). For this, PDA culture medium supplemented (9:1) with sterile solution of reconstituted skim milk powder (10.0%) was prepared. Subsequently, the medium was poured into Petri dishes, onto which a representative of each morphospecies was inoculated. The plates were incubated at 25 ± 3°C for five days. To verify the effect of temperature on enzymatic activity, the plates were also incubated at two additional temperatures: 28±3 ºC and -4±3 ºC for five days. All experiments were performed in duplicate. After the end of the incubation period, proteolytic activity was analyzed through the presence of a translucent halo, indicating positive proteolytic activity, while its absence indicated negative proteolytic activity.

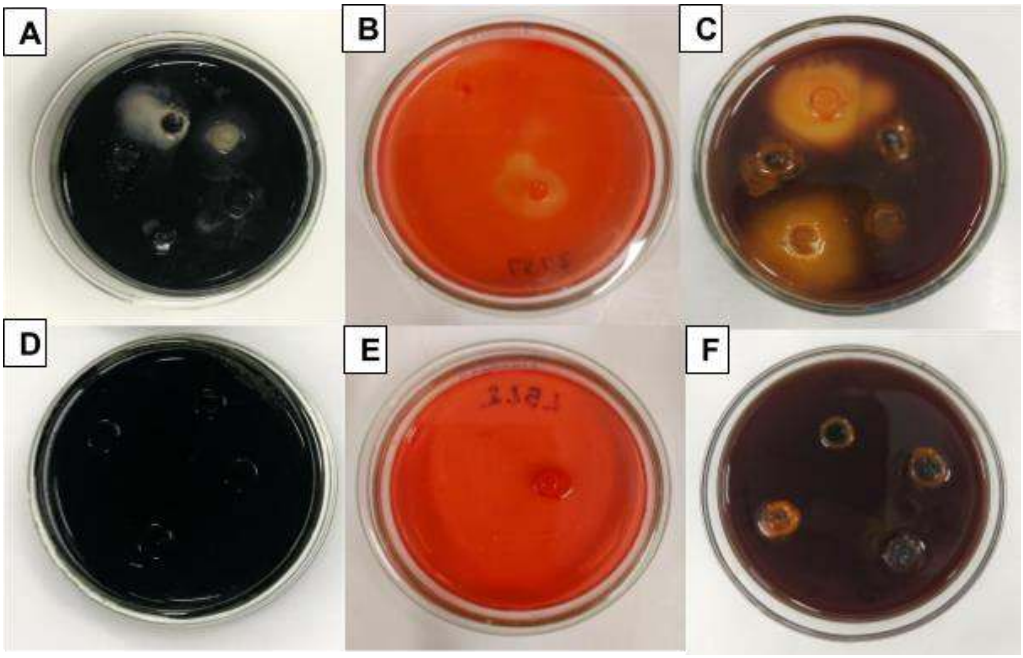
3 RESULTS AND DISCUSSIONS

In total, three yeasts were isolated from lobeira (*S. lycocarpum*), designated LC1, LC2 and LP1. Overall, 18 yeast strains were used in the enzymatic activity screening tests (15 mentioned in Table 1 and three from lobeira fruits).

Enzymatic activities were detected by observation of Petri dishes through the formation of halos, as well as by measurement and calculation of the mean enzymatic activity indices (EAI). Figure 1 illustrates plates with positive and negative results for the tested enzymes.

Figure 1

Halo (clear zone around the fungal colony) indicating positive enzymatic activity in the screening of microorganisms in the production of (A) amylase, (B) cellulase and (C) Pectinase and negative enzymatic activity for the respective enzymes (D),(E), (F)



Source: Authors (2023).

In Table 2, the percentages of yeasts producing cellulase, amylase, protease and pectinase among the 18 strains tested are presented.

Table 2

Percentage of yeasts positive for the enzymatic activity tested

Test performed	Visualization method	Number of positive yeasts/total yeasts tested	% of total
Celulase	Staining with Congo red solution	8/18	44,44
Amilase	Staining with Lugol's solution	5/18	27,77
Protease	Naked eye visualization	0/18	0.00
Pectinase	Staining with Lugol's solution	4/18	22.22

Source: Authors (2026).

The results of the EAI calculations for amylolytic, cellulolytic, and pectinase activities are presented in Table 3. No proteolytic activity was observed among any of the tested yeasts.

Table 3

Enzymatic activity indices (EAI) of amylase, cellulase and pectinase of yeast isolates

Isolated	EAI (mm)

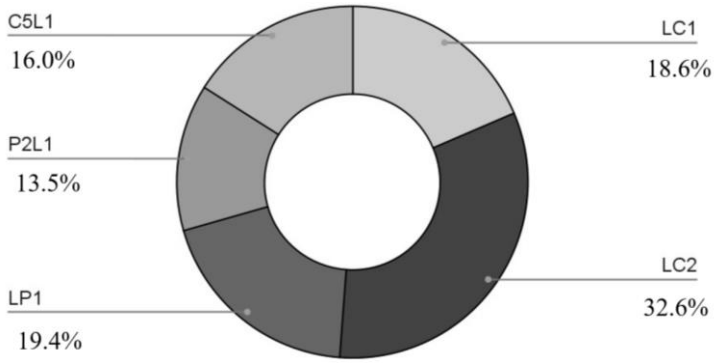
	Amilase	Celulase	Pectinase
LC1	1,38	1,00	1,89
LC2	2,42	1,01	1,33
LP1	1,43	1,20	0
C5L1	1,18	0	0
P1L1	0	1,28	0
P2L1	1,0	1,68	1,36
P3L1	0	1,74	0
P4L1	0	0,99	0
L1L10	0	1,03	0
M217	0	0	1,38

Source: Authors (2026).

From the determination of the EAI, according to the data presented in Table 3, it was possible to observe that the LC2 yeast stood out of the amylase production, as illustrated in Figure 2.

Figure 2

Analysis of the averages of enzymatic activity of amylases



Source: Authors (2026).

In relation to the analysis of the cellulolytic activity assay, the yeasts were tested at different pH ranges, at fixed temperature (25 ± 3 ºC). As a result, it was observed that 44.44% of the yeasts showed activity in alkaline pH medium, 27.77% in neutral medium, and 22.22% in acidic medium. The EAI results obtained for each pH variation are found in Table 4 and Figure 3. It was verified that the EAI in acidic medium was significantly higher compared to those obtained for the other pH conditions, thus confirming the influence of pH on the detection of cellulolytic activity.

Table 4

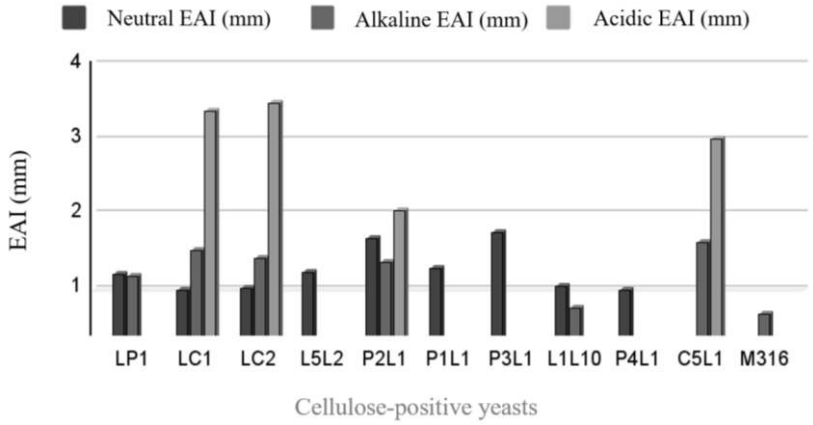
Averages of EAIs of cellulolytic activity (with and without pH variation)

Yeasts positive for cellulase	Origin	EAI Neutral (mm)	EAI Basic (mm)	EAI Acidic (mm)
LP1	*S. lycocarpum*	1,20	1,17	0
LC1	*S. lycocarpum*	0,98	1,5	3,37
LC2	*S. lycocarpum*	1,01	1,4	3,48
L5L2	*S. frugiperda*	1,22	0	0
P2L1	*A. occidentale*	1,68	1,35	2,05
P1L1	*C. brasiliense*	1,28	0	0
P3L1	*A. occidentale*	1,74	0	0
L1L10	*S. frugiperda*	1,03	0,75	0
P4L1	*A. occidentale*	0,99	0	0
C5L1	*A. occidentale*	0	1,62	3,0
M316	*Zea mays*	0	0,66	0

Source: Authors (2026).

Figure 3

Analysis of cellulase enzymatic activities of yeasts with pH variation

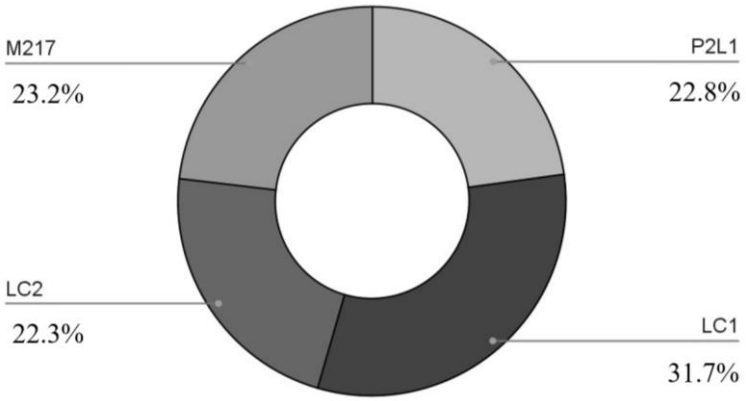


Source: Authors (2026). Prepared by the author from data obtained experimentally.

Regarding the pectinolytic activity assay, 22.22% of the environmental yeasts tested formed a pectin degradation halo in the culture medium, indicating that these strains were capable of producing pectinases. The LC1 isolate showed the highest average EAI, although this value was lower than 2 (EAI = 1.89), as illustrated in Figure 4.

Figure 4

Analysis of the averages of pectinolytic activity



Source: Authors (2026).

In the literature, most authors recommend that microorganisms presenting EAI ≥ 2.0 be considered enzyme producers in solid medium (CESKA, 1971; LIN et al., 1991). According to the data presented in Table 3, only the LC2 isolate showed amylolytic activity greater than 2 (EAI = 2.42). It was observed that 55.55% of the strains were able to produce at least one of the three enzymes evaluated; however, with EAI values lower than 2.0. The LC1, LC2 and P2L1 isolates stood out for producing three of the four enzymes tested, indicating the potential of these microorganisms for enzyme production, even though their EAI values are below the recommended treshold.

The quantitative determination of a specific enzyme present in a medium is complex, since fractions of the enzyme may be inactive or only partially active. Thus, the assessment of enzymatic activity becomes the most relevant parameter to be considered in enzyme quantification (CHAPLIN et al., 1992).

When dealing with amylolytic activity by yeasts, the EAI values observed are generally lower compared to those produced by filamentous fungi, even under the same culture conditions (medium composition, temperature, and pH), which demonstrates instrinseic differences between the activity of filamentous fungi and yeasts (PEIXOTO, 2006; ALVES, 2017). The factors that influence reduced amylolytic activity include: carbon source, nitrogen source, phosphate concentration, as well as the pH and temperature of the medium. Studies show that when the medium is supplemented with maltose in addition to starch, there is a significant increase in enzymatic activity (SPIER, 2005). The production of amylase by yeasts also shows substantial improvement when inorganic salts, such as ammonium sulfate and ammonium nitrate, are used as a nitrogen sources (GUPTA et al., 2003). The pH and temperature, in turn, are determining parameters in all enzymatic production processes; however, fungal systems, buffering components of the culture medium may reduce the need

for strict pH control. The temperature applied in this study falls within the optimal range for the amylase production, which is reported to be approximately 25 to 37ºC.

The LC2 yeast, obtained from the peel of the lobeira fruit, expressed 32.6% enzymatic activity, representing the highest index observed in the study. Further studies are required to optimize the psysical and chemical conditions in order to enhance the production of the enzyme evaluated, as well as to conduct extraction, quantification, characterization, and precipitation analyses of the enzymatic extract obtained.

In relation of the analysis of the cellulolytic activity, Table 4 shows that higher cellulolytic activity (EAI ≥ 2.0) was observed for the yeasts LC1, LC2, P2L1 and C5L1 in acidic medium. In the other media tested, no significant difference was observed among the mean EAI values, as illustrated in Figure 03. The yeast isolates M316, P4L1, L1L10, P3L1, P1L1, L5L2 and LP1 did not exhibit cellulolytic activity under acidic pH conditions. The M316 isolate showed positive activity only in basic medium, although with low EAI values. These results allow the inference that the optimal pH for cellulase production under the conditions evaluated is approximately 4.0 for the LC1, LC2, and C5L1 isolates. For the remaining isolates, no definitive conclusions can be drawn, although an increase in pH appears to promote higher enzymatic activity in some strains.

One of the notable characteristics of yeasts is their ability to grow over a wide range of acidic pH values (JAY, 1992). Amylases exhibit moderate resistance to alkaline pH; however, they are capable of acting over a broad temperature range (PEIXOTO, 2006). Studies show that amylase present optimal activity between pH ranges of 4.4 to 5.5 with temperatures of up to 60 º C. At pH values above than 7.5 the activity of this enzyme is minimal or abasent within the same temperature range (WANDERLEY et al., 2004; STAMFORD et al., 1998; CORDEIRO et al., 2002).

Considering the results presented in Table 4 and Figure 3, the screening results for cellulase-producing yeasts are consistent with those reported in the literature. However, more detailed studies on culture medium composition, as well as process parameters such as temperature, aeration, among others, are required to optimize the enzyme production by the yeasts that exhibited higher cellulolytic activity.

In this study, 22.22% of the environmental yeasts exhibited pectinolytic activity. Couto (2008) conducted a study with yeasts obtained from agroindustrial residues for the pectinase production, verifying that most of the yeast species tested produced varying amounts of pectinases. Alves (2017) tested the biotechnological potential of bacteria and yeasts isolated from green coconut peel *(Cocos nucifera)* during fermentation. The results of enzymatic activity assays showed that one bacterial isolate was able to produce pectin, and that 22

tested yeasts isolates were capable of degrading pecting, with WAI values around 2.26. In general, fruits and vegetables are rich in pectic substances, which confer important biological functions, such as cell signaling, adhesion, proliferation and strength and flexibility to the cell wall, among others (ALVES, 2017). Therefore, higher pectinolytic activity was expected among the yeasts evaluated in this study.

In the tests performed to verify proteolytic activity, all yeasts evaluated showed negative results, even with the variation in incubation temperature. Although the production of protease by yeasts has long been the subject of investigation, few studies report significant proteolytic activity in these microorganisms. Rodarte (2005) tested 38 yeasts isolates for protease production, obtaining a positive result in only one isolate.

In the medical field, proteases are studied mainly in pathogenic yeasts, such as *Candida albicans*, because this species has the ability to secrete extracellular aspartic-protease important in its pathogenic nature. Thus, the development of studies of proteolytic enzymes developed by *Candida albicans* have contributed to the development of drugs for the treatment of candidiasis (KOELSCH et al., 2000). Studies involving yeasts from different *Candida* species have also been conducted with a focus on industrial applications, demonstrating positive results in the hydrolysis of casein (RODARTE, 2005).

Although no proteolytic activity was detected in the yeasts evaluated, this does not preclude the development of further studies, given the commercial, medical, and industrial importance of proteases. Methodological adjustments or the prospecting of new environmental sources may represent key strategies for the discovery of novel isolates with biotechnologically relevant characteristics.

4 CONCLUSION

It was concluded that yeasts with biotechnological potential, such as the LC2, LC1, and P2L1 isolates, can be obtained from environmental substrates, especially edible fruits and pseudofruits. The highest enzymatic activity indices (EAIs) were observed for the LC2 isolate, which exhibited the best cellulolytic activity under acidic pH conditions. Future studies should focus on the taxonomic characterization of these yeasts, as well as on evaluating their potential for the production of other enzymes of biotechnological interest, such as lipases and glucose oxidase, among others. Studies such as this reinforce the importance of conserving and valuing the biodiversity of the Cerrado biome.

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