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Production of amylase by *Saccharomyces cerevisiae* yeast

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ABSTRACT

Currently, there are several studies looking for new sources of enzymes, mainly through the use of microorganisms. As a result of these searches, some yeast strains have been studied to verify the potential production of amylolytic enzymes, due to their wide applicability. The aim of this study was to identify different strains of *Saccharomyces cerevisiae* capable of producing amylolytic enzymes through submerged fermentation enriched with 1% starch. Seven strains were tested using plate screening methodology, of which 3 showed better capacity for amylase production and were used in submerged fermentation, obtaining activities of: SC52 (4.63 U/mL), SC37 (2.12 U/mL), and SC82 (1.23 U/mL). Subsequently, for the best strain, a Box-Behnken statistical design using response surface methodology was applied to optimize the culture parameters (starch concentration, pH, time, and temperature) of the fermentation processes for amylase production. Overall, SC52 showed better amylase production capacity, and adjustments to the design should be made to optimize fermentation conditions, aiming for an increase in amylolytic enzyme production.

Keywords: Yeast. Submerged fermentation. Enzyme. Biotechnology. Microorganism.

1 INTRODUCTION

Yeast is one of the microorganisms that play an important role in the fermentation process of food[1]. Among the yeasts used in industrial fermentation processes, *Saccharomyces cerevisiae* stands out as the dominant yeast compared to other yeasts used. In addition to their use in numerous industrial processes, yeasts of the genera *Candida glabrata*, *Pichia anomala* have gained prominence as potential producers of amylase enzymes responsible for the degradation of starch to sugars[2].

Among the amylolytic enzymes, amylase stands out. It belongs to the group of hydrolase enzymes that catalyze biological hydrolysis reactions using water (H_2O) [3,4]. This enzyme acts by randomly breaking the α-1,4-glycosidic linkages in starch, especially in long chains, to produce maltotriose and maltose from the amylose polymers in starch and to produce glucose and some dextrin from the amylopectin polymer that makes up starch, as well as other organic acids. Its ability to randomly hydrolyze glyosidic linkages makes it faster than other amylases, such as β-amylase [5]. Amylases are required in various industries: textile industry, food production (beer, bread, syrup, artificial sweeteners, animal feed industry), ethanol production, detergents, pharmaceuticals and enzyme supplements [6].

Although it is widely used in various industrial processes, there are no recent studies on the production of amylolytic enzymes by *Saccharomyces cerevisiae*. Therefore, the present study aims to investigate different strains of *Saccharomyces cerevisiae* isolated from cachaça fermentation tanks to determine if these strains have the ability to produce amylolytic enzymes.

2 MATERIAL & METHODS

Yeast Screening for Amylase Production: The yeasts used were isolated from cachaça fermentation tanks in Bahia and molecularly identified by Silva [7]. The yeasts were reactivated on YPD agar and incubated in a BOD chamber (30°C/24 h). After this time, 0.8 cm diameter discs were cut from the plates containing yeasts and placed on plates containing Czapeck medium + 1% starch and again incubated in a BOD chamber (30 °C/24 h). The enzymatic activity of amylase was observed by the formation of a starch hydrolysis halo using a 1% iodine solution after 24 and 48 hours of growth. The yeast with the highest hydrolysis rate was selected for submerged fermentation.

Selection of strains for amylase production by submerged fermentation: Fermentation was carried out in 125 mL Erlenmeyer flasks with 25 mL Czapeck medium containing (g/L): (K_2HPO_4 (1.0 g/L), $MgSO_4$ (0.5 g/L), KCl (0.5 g/L), $NaNO_3$ (3.0 g/L)) with the addition of 1% soluble starch. The culture medium was then sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the medium was cooled for subsequent yeast inoculation. Inoculation was performed at 1-2 x 10^7 cells/mL. The culture was maintained at 30 °C with constant shaking at 150 rpm for 24 hours. After this period, the culture was centrifuged at 12.000 *g* for 15 minutes at 4 °C and the cell-free supernatant was considered as crude enzyme extract, collected and used for subsequent analyses.

Determination of Amylase Activity: Enzyme activity was determined by adding 125 µL of enzyme extract, 125 µL of 1% sodium phosphate buffer, and 125 µL of 100 mM sodium phosphate buffer (pH 7.0). After a 10 minute reaction at 50 °C, the reducing sugar released was quantified by the 3,5-dinitrosalicylic acid (DNS) method [8]. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µmol of product per minute of reaction.

Optimization of Amylase Production: After selecting the best amylase-producing strain, a three-factor, three-level Box-Behnken design was used to optimize amylase production. The independent variables were Starch concentration (1, 5, and 9%); Time (24, 48, and 72 hours); and pH (4, 6, and 8), with enzyme activity (U/mL) as the dependent variable.

3 RESULTS & DISCUSSION

Qualitative Amylolytic Activity Test

Plate screening was performed to identify yeasts capable of producing amylolytic enzymes to hydrolyze starch and to show which isolates were able to utilize soluble starch as the sole carbon source. Among the isolates, all showed starch hydrolysis. Although there were no significant differences among them, strains SC52, SC37, and SC82 were selected because they showed better activity in the bench tests. Table 1 shows the average halos for starch degradation.

Table 1 Halos resulting from the hydrolysis of starch by yeast

Isolated	SC37	SC52	SC82	L3/63	L67	L10	L3/65
Average halos (cm)	1.2 ± 0.17[a]	1.4 ± 0.15[a]	1.3 ± 0.17[a]	1.1 ± 0.15[a]	1.1 ± 0.15[a]	0.9± 0.12[a]	1.1 ± 0.15[a]

* Means followed by the same letter are not significantly different according to Tukey's test (P < 0.05).

Plate agar screening has been widely used for the discovery of new enzymes, especially in metagenomic screening, due to its simple, direct and efficient approach based on enzymatic functions, which can lead to the discovery of new genes related to starch hydrolysis and other enzymatic activities [9].

Although yeasts are commonly used vectorially to express the amylase secretor gene derived from genes of bacteria and filamentous fungi, wild yeasts have attracted attention for their ability to utilize starch, which is the most abundant biomass after cellulose as a carbon source [10]. While amylase production by the genus *Saccharomyces* is less common, this study demonstrated that strains of *Saccharomyces cerevisiae*, particularly SC52, were able to produce starch-degrading halos. After the plate screening technique, the top three strains were selected and subjected to submerged fermentation. After fermentation, it was observed that the yeasts exhibited significant amylase activity when starch was used as the carbon source, as shown in Figure 1.

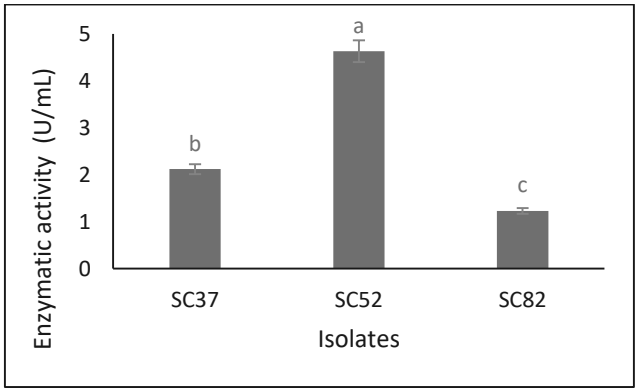

Figure 1. Amylase activities produced by submerged fermentation of isolates SC37, SC52, and SC82
*Means followed by the same letter are not significantly different by Tukey's test (P < 0.05).

All isolates showed good enzymatic activity considering that yeasts generally have lower enzymatic activity compared to bacteria and filamentous fungi. Among the yeasts, SC52 stood out with an enzymatic activity of (4.63 U/mL), followed by SC37 (2.12 U/mL) and SC82 (1.23 U/mL). Ogden and Tubb [11], who studied amylase production by *Saccharomyces cerevisiae* CBS 2514, obtained a maximum activity of (1.50 U/mL) after 66 hours of fermentation, while for the tested yeasts we obtained a higher activity after 24 hours, indicating their potential for amylase production and emphasizing the need for an optimization study of amylase production for SC52.

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The response surface plot (Figure 2) was generated from the experimental data to delineate the optimal region for enzyme production. It can be concluded that with the data used, it was not possible to determine the optimal region of enzymatic activity, so some adjustments in the design were necessary to achieve the maximum region in an overall more efficient manner.

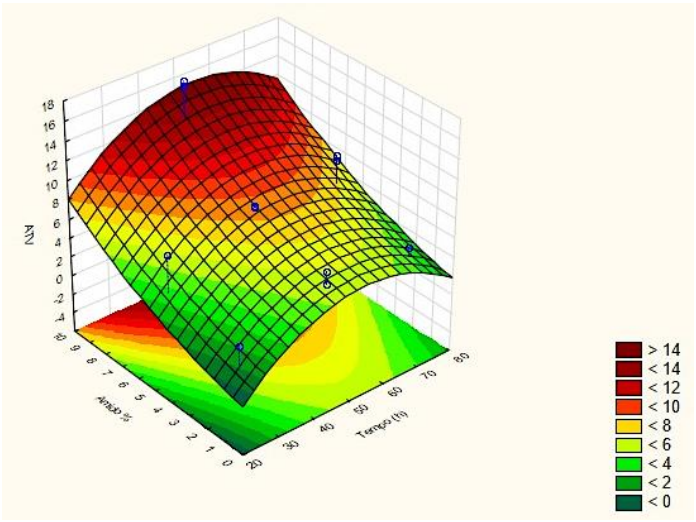

Figure 2. Response surface of the effects of the combination of starch concentration and time on the production of SC52 amylase.

4 CONCLUSION

It can be concluded that the *Saccharomyces cerevisiae* yeast evaluated has the potential for amylase production. The enzymes exhibited high structural stability and maintained their catalytic potential at different pH levels and temperatures. Therefore, further optimization experiments are necessary to evaluate the maximum capacity for amylase production. Based on the aforementioned characteristics, it can be posited that these enzymes have significant biotechnological potential.

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