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Exopolysaccharide biosynthesis by a newly-isolated *Cryptococcus laurentii* SD7 in sugar cane molasses and inorganic nitrogen sources

Biossíntese de exopolissacarídeo por um novo isolado de *Cryptococcus laurentii* SD7 em melaço de cana-de-açúcar e fontes inorgânicas de nitrogênio

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Microbial polysaccharides are of great biotechnological and industrial importance due to their functional properties and wide range of applications in the food, cosmetic and pharmaceutical industries. For the microbial synthesis of polysaccharides, f by-products or agro-industrial residues has been widely used as alternative substrates to reduce production costs. In this study, we evaluated several sources of carbon (sucrose, lactose, glucose, glycerol and sugarcane molasses at 5%) and nitrogen (organic and inorganic at 1%) as substrates for the biosynthesis of exopolysaccharides (EPS) by *Cryptococcus laurentii* SD7. The best substrates were sugarcane molasses and the combination of $(NH_4)_2SO_4$ and $NaNO_3$ salts. To study the effect of combining these substrates and optimize EPS production, the Central Composite Design (CCD) 2^3 methodology was used, resulting in 17 trials, where the concentration of sugarcane molasses varied from 1% to 9% and nitrogen sources varied from 0.2% to 0.8%. The factorial design showed that $NaNO_3$ did not affect EPS production, but sugarcane molasses and $(NH_4)_2SO_4$ were effective at concentrations of 9% and 0.2%, respectively, reaching EPS production of 11.0 g L^{-1}. The results were very promising, showing that *C. laurentii* SD7 has high potential for EPS production on low-cost substrates.
Key-words: biopolymers, fungi, agro-industrial by-products.

Polissacarídeos microbianos são de grande importância biotecnológica e industrial devido às suas propriedades funcionais e ampla gama de aplicações nas indústrias alimentícia, cosmética e farmacêutica. Para a síntese microbiana de polissacarídeos, subprodutos ou resíduos de origem agroindustrial tem sido muito utilizados como substratos alternativos para redução dos custos de produção. Neste estudo, foram avaliadas várias fontes de carbono (sacarose, lactose, glicose, glicerol e melaço de cana-de-açúcar a 5%) e nitrogênio (orgânicas e inorgânicas a 1%) como substratos para a biossíntese de exopolissacarídeos (EPS) por *Cryptococcus laurentii* SD7. Os melhores substratos foram o melaço de cana-de-açúcar e a combinação dos sais $(NH_4)_2SO_4$ e $NaNO_3$. Para estudar o efeito da combinação destes substratos e otimizar a produção de EPS foi utilizada a metodologia de Delineamento Composto Central Rotacional (DCCR) 2^3 que resultou em 17 ensaios, onde a concentração de melaço de cana-de-açúcar variou de 1% a 9% e das fontes de nitrogênio variou de 0,2% a 0,8%. O delineamento fatorial mostrou que $NaNO_3$ não afetou a produção de EPS, mas o melaço de cana-de-açúcar e o $(NH_4)_2SO_4$ foram eficazes nas concentrações de 9% e 0,2%, respectivamente, atingindo produção de EPS de 11,0 g L^{-1}. Os resultados foram bastante promissores, mostrando que *C. laurentii* SD7 tem alto potencial para produção de EPS em substratos de baixo custo.
Palavras-chave: biopolímeros, fungos, subprodutos agroindustriais.

1. INTRODUCTION

Microbial exopolysaccharides (EPS) are complex carbohydrate polymers produced by various microorganisms like bacteria, fungi, and yeasts, and secreted outside the cell. EPS are high-value biomaterial with biotechnological and commercial potential wide range of applications in the food, cosmetics, and drug industries. EPS has diverse biological roles, such as emulsifiers, thickeners, flocculants, stabilizers, antioxidants, antimicrobials, anticoagulants, antitumors,

anticholesterolemics, and immunomodulators, owing to their molecular weight and chemical structure [1-3].

Microbial EPS has some advantages over traditional polysaccharides obtained from plants and algae, such as the degree of purity, high polymerization potential, and greater water retention capacity. Additionally, their microbial production is more advantageous because of the ease of cultivation and control of the physicochemical parameters involved in their production [4, 5]. Moreover, the process of extracting microbial EPS is usually simpler and faster because of the short life cycle of microorganisms.

EPS can bind to the cell forming a capsule or be secreted into the extracellular medium in the form of slimes, increasing the viscosity of the fermentation medium throughout the fermentation process [6]. However, a more viscous media disturbs the distribution of oxygen and nutrients in the culture, hindering microbial metabolism. For commercial processes, media with less pseudoplastic and more Newtonian characteristics are more desirable; therefore, to reduce the interference of undesirable factors, the physical and chemical parameters are regulated by the means of processes, such as agitation, aeration, and C:N ratio adjustment [3, 6]. Though not essential for survival, EPS provides microorganisms protection against phagocytosis, osmotic stress, desiccation, and biofilm formation, and many are also associated with microbial virulence [7, 8]. Thus, they are considered secondary metabolites produced under adverse conditions, such as excess or scarcity of nutrients, low temperatures, or acidic pH [6, 9].

Despite the wide applicability of microbial EPS in the various industrial sectors, the cost-benefit of these bioprocesses has limitations, as some microorganisms do not produce EPS in sufficient amounts, and the traditional carbon sources used, such as glucose and sucrose, increase the cost of the process. Therefore, different studies aimed at the use of agro-industrial substrates for the production of biomolecules of interest, such as EPS and Single Cell Protein [10-13]. The use of these substrates is a sustainable alternative, as this adds value to the agro-industrial by-products that are often discarded and can reduce environmental contamination problems [10, 14, 15] closing the loop of the circular economy or “green” economy. Among the agro-industrial substrates, sugar cane molasses is a very viable alternative, as it is a renewable source, is produced on a large scale, and contains sugars, vitamins, minerals, and amino acids that can satisfactorily replace the use of conventional sources [16-19]. Furthermore, low-cost nitrogen sources, such as inorganic salts and agro-industrial waste, are also interesting for reducing costs.

The aim of the present study was to examine potential of several carbon sources, including agro-industrial byproduct, and different nitrogen sources as nutrient in place of conventional media components by *Cryptococcus laurentii* SD7, a yeast isolated from a freshwater mollusk.

2. MATERIAL AND METHODS

2.1 Fermentation condition and analytical methods

Yeast was isolated from a freshwater-bivalve mollusk, *Mytella guyanenses*, and was identified by molecular biology techniques as *Cryptococcus laurentii* SD7 [19]. After screening with several yeast strains, C. *laurentii* SD7 was selected for its higher capacity to produce exopolysaccharides. The yeast was incubated on Sabouraud dextrose agar for 24 at 28 °C. The fermentation experiments were carried out in 125 mL Erlenmeyer flasks containing 25 mL composed (%): $(NH4)_2SO_4$ 0.2; KH_2PO_4 0.1; $MgSO_4.7H_2O$ 0.05; $CaCl_2$ 0.01; NaCl 0.01; yeast extract 0.1. The inoculum consisted of 10% of the fermentation medium volume after 24 hours of growth, different carbon at 5% and nitrogen sources at 1% (Figure 1 and Table 1). The culture was incubated for 96 h with continuous agitation at 150 rpm at 25 ± 2 °C. Then culture was centrifuged at 5,000 rpm for 20 minutes at 4°C. Supernatant was utilized for EPS determinations and biomass for cellular growth. The results obtained were analyzed using one-way analysis of variance (ANOVA) with Tukey test (carbon sources) and Scott-Knott test (nitrogen sources) at 5% significance ($p<0.05$).

2.1.1. EPS determination

Whole cell cultures were centrifuged at 5,000 rpm for 20 min. to separate the yeast cells from the supernatant. The exopolysaccharides in the cell-free supernatant were precipitated with 2 vol. of 99% ethanol at 4ºC for 24 hours. The resultant supernatant was discarded, and the pellet was dried at 80ºC until constant weight and weighed.

2.1.2. Cellular growth determination

The biomass in the culture was pelleted by centrifugation at 5,000 rpm for 20 min, washed twice with distilled water, and then dried at 80 ºC until constant weight.

2.2 Optimization of EPS production by Response Surface Methodology

The optimization of EPS production was performed by Response Surface Methodology using Central Composite Design (CCD). EPS production (g L^{-1}) was the dependent variable and sugarcane molasses, sodium nitrate ($NaNO_3$), and $(NH_4)_2SO_4$ were set as the independent variables. A CCD matrix was comprised of a total of 17 experimental runs with three levels (-1, 0, +1) including three replicates at the central point and two axial points (+1.68 and −1.68) according Rodrigues and Iema (2009) [20] (Table 3). This model was represented by a second-order polynomial equation (Equation 1):

$$Y = b_0 + b_1.X_1 + b_2.X_2 + b_3.X_3 + b_{12}.X_1.X_2 + b_{13}.X_1.X_3 + b_{23}.X_2.X_3 + b_{11}.X^2 + b_{22}.X^2 + b_{33}.X^2 \quad \text{(Eq. 1)}$$

where: Y is the predicted response of EPS production; X_1, X_2, and X_3 are the encoded forms (molasses, $NaNO_3$, and $(NH_4)_2SO_4$, respectively); b_0 refers to the intersection point; b_1, b_2, and b_3 are linear coefficients; b_{12} is the double interaction coefficient; and b_{11}, b_{22}, and b_{33} are the quadratic coefficients.

The producing fermentation medium comprised (%): KH_2PO_4 0.1; $MgSO_4.7H_2O$ 0.05; $CaCl_2$ 0.01; and NaCl 0.01. The concentration of sugarcane molasses, $(NH_4)_2SO_4$, and $NaNO_3$ were determined according to Table 3. The results obtained from the experimental model were analyzed in Statistica version 7.1®.

3. RESULTS AND DISCUSSION

3.1 Effect of different carbon sources on EPS and biomass production

Seven carbon sources at a concentration of 5% were investigated to identify the optimal carbon source for EPS production. Sugar cane molasses was found to be the most suitable in improving EPS production, 7.5 g L^{-1} after 96 h, showing a productivity of approximately 0.08 g L^{-1} h^{-1}. This production was, on average, 2.5 times greater than that of sucrose, glucose, lactose, and maltose each, which did not present any significant statistical differences, producing on average, 3.2 g L^{-1} of EPS. Glycerol was the worst substrate for EPS production, with 2.0 g L^{-1} (Figure 1). Consequently, sugar cane molasses was chosen for further studies as carbon source.

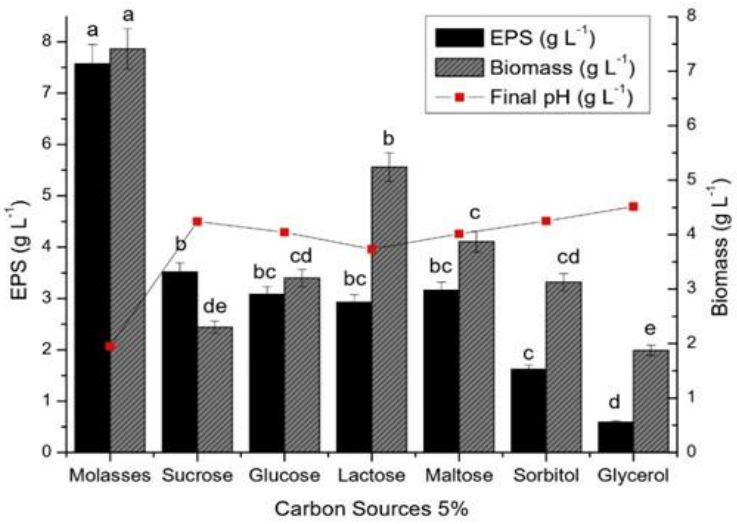

Figure 1: Effect of different carbon sources in the EPS and biomass production by Cryptococcus laurentii SD7. Equal letters do not differ from each other by Tukey's test at 5% significance ($p<0.05$).

Studies reporting EPS production by yeasts using sugarcane molasses are scarce; however, in bacteria are common and, in general, molasses positively affected and promoted higher and better EPS synthesis compared to simple carbon sources, such as sucrose, glucose, lactose, and maltose [10, 21-25]. Among the simple sources of carbon, sucrose stood out in many works, using different species of bacteria as *Chryseobacterium indologenes* MUT2, *Stenotrophomonas maltophilia, Brevibacillus parabrevis, Paecilomyces hepiali* HN1 and *Phellinusvaninii* Ljup [26-29].

These results suggested that the EPS production was increased by the presence of salts such as phosphates, sodium, calcium, magnesium and phosphorus and several amino acids. Another important factor was the high content of fermentable sugars in molasses, such as sucrose, glucose, and fructose. This combination of macroelements and microelements likely contributed to improving EPS production, making molasses a sustainable alternative substrate [30, 31].

In the yeasts, most works described sucrose as the best carbon source for EPS production in different species. *Sporobolomyces salmonicolor*, *Cryptococcus albidus*, *C. laurentii*, and *Cryptococcus* sp. (up to 5.2 g L^{-1},168 h) [32]. Pavlova et al. (2009) [33] studied EPS production by three yeast isolates (AL49, AL51 and AL54), three strains of the *Cryptococcus* sp., *Rhodotorula minuta* AL1, and *Debaryomyces hansenii* AL3, which achieved the highest EPS yields with sucrose (up to 3.04 g L^{-1}, 120 h). Similarly, Silambarasan et al. (2019) [34] and Hamidi et al. (2020) [35], using *Rhodotorula* sp. CAH2 and *R. mucilaginosa* sp. GUMS16, also describe sucrose as best carbon source. This may explain the higher yield of *C. laurentii* SD7. The molasses composition contained between 50% and 70% total sugars, with sucrose being the main sugar, representing between 30% and 40%. Furthermore, the molasses had a nitrogen content between 0.5% and 0.9% [29, 31, 36, 37].

However, the highest EPS production is not associated only with the carbon source but also with the microbial species, which changes the enzymatic apparatus [38], showing that generalizations are not possible for yeasts. Gientka et al. (2016) [39] reported that maltose was the best carbon source for EPS production *Candida famata* and *C. guilliermondii*, which produced twice as much when compared with sucrose. Furthermore, they reported that lactose was not assimilated by *C. guilliermondii* for EPS production and showed a low yield by *C. famata.* On the other hand, in this work *C. laurentii* SD7 obtained good production with lactose. Samadlouie et al. (2020) [38] produced the highest amounts of EPS using starch, followed by sorbitol by the yeast *Rhodotorula minuta* ATCC 10658. These results differed from the present work, where sorbitol showed one of the lowest EPS yields (Figure 1). Gientka et al. (2016) [39] also described the low production of EPS using sorbitol and glycerol, suggesting that these substrates are unfavorable for some species.

Molasses was also the most efficient carbon source for biomass production by *C. laurentii* SD7, reaching 7.86 g L^{-1}. These results were similar to those described by Srikanth et al. (2014) [21] with *Aureobasidium pullulans* MTCC 2195, as molasses provided sufficient energy and nutrients for good yields of both biomass and EPS. According to Xu and Xu (2014) [30] and Soukoulis and Tzia (2018) [31], sugar cane molasses has essential amino acids in its composition that favor microbial growth. The cell growth is not directly related to EPS production, as it normally occurs when the microorganism is in unfavorable conditions for its growth [40, 41]. Generally, at the end of the fermentation, was observed a decrease in growth and an increase in EPS synthesis by the microorganism [22]. For *C. laurentii* SD7, the combination of sugar cane molasses and corn-steep liquor ceased the cell growth after 48 h, but EPS production increased until 198 h [19]. These results suggested that the culture medium had enough nutrients to provide energy and promote cell growth. However, some components of the medium, such as the excess of nutrients and the acidic pH, may also have favored EPS production as a mechanism of protection [22, 42].

A typical characteristic of EPS production is the variation in pH over time [28, 32, 39, 43-45]. In the tests with *C. laurentii* SD7, less acidification of the culture medium was observed with the simple carbon sources (initial pH 5.5 and final pH between 4.0 - 4.5). On the other hand, a much more pronounced acidification occurred with cane molasses, and final pH reached 2.0, which favored EPS production (Figure 1).

According to Poli et al. (2010) [43] and Ergene and Avci (2017) [22], the pH becomes more acidic as EPS production increases because of a feedback process. Some EPS have organic acids in their chemical structure, such as glucuronic acid, which can contribute to a decrease in the pH of the medium when EPS is produced in large quantities [42, 46].

3.2 Effects of different nitrogen sources on EPS and biomass productions

Five nitrogen sources at a concentration of 1%, as well as the combination between them, were investigated for EPS and biomass production by *Cryptococcus laurentii* SD7 was carried out using 5% molasses as a carbon source (Table 1).

Table 1: Effect of different nitrogen sources on EPS and biomass production by Cryptococcus laurentii SD7.

Assay	Nitrogen source (1%)	EPS (g L^{-1})	Biomass (g L^{-1})	Final pH
1	$NaNO_3$ + $(NH_4)_2SO_4$	8.03[a]	5.47[e]	2.0
2	Yeast extract + $(NH_4)_2SO_4$	7.30[b]	9.82[c]	2.2
3	$(NH_4)_2SO_4$	7.27[b]	7.28[d]	1.6
4	$NaNO_3$ + Peptone	7.04[b]	12.83[b]	4.0
5	$NaNO_3$ + Yeast extract	6.73[b]	13.70[b]	3.7
6	Yeast extract	6.66[b]	13.63[b]	3.9
7	Peptone	6.22[c]	15.18[a]	4.2
8	Peptone + $(NH_4)_2SO_4$	5.40[d]	6.99[d]	2.0
9	Peptone + Yeast extract	5.28[d]	12.62[b]	4.2
10	Urea + $(NH_4)_2SO_4$	4.22[e]	16.75[a]	6.8
11	Urea + Yeast extract	3.26[f]	11.43[b]	6.9
12	Urea + Peptone	3.11[f]	9.82[c]	5.9
13	$NaNO_3$	3.01[f]	4.98[e]	6.1
14	Urea + $NaNO_3$	2.97[f]	9.97[c]	7.1
15	Urea	2.62[f]	15.75[a]	6.9

Means followed by the same letters do not present statistical difference by Scott-Knott test at 5% probability ($p<0.05$).

According to the results, inorganic nitrogen sources were more favorable for EPS and biomass production compared to organic nitrogen sources. High EPS productions were obtained with combination between yeast extract, peptone, $NaNO_3$, and $(NH_4)_2SO_4$ each other, reaching above 7.0 g L^{-1} (assays 2 to 4, Table 1). However, the highest production was achieved in the combination of $NaNO_3$ + $(NH_4)_2SO_4$, 8.0 g L^{-1} (assay t 1, Table 1).

On the other hand, pure urea or in combination with $(NH_4)_2SO_4$ were the best for the biomass production reaching, on average, 16.0 g L^{-1} (assay 10). These results showed statistically similar values to peptone (assay 7), proving to be a good alternative of nitrogen source for the single cell protein production.

The use of culture media containing inorganic salts represents a significant cost reduction in the process. Compared to complex nitrogen sources such as yeast extract and peptone, this can represent savings of about 10 times or more. In addition, in the combination of $(NH_4)_2SO_4$ and $NaNO_3$, the experimental results showed excellent repeatability. Furthermore, the EPS produced showed stability after drying, presenting a white color and a hard and resistant aspect. Wu et al. (2008) [47] and Goyzueta et al. (2020) [48] confirmed that there is variation in the composition of EPS according to the nitrogen source, affecting the final stability and functionality. Therefore, for the next experiments, the nitrogen sources selected were $(NH_4)_2SO_4$ and $NaNO_3$.

In general, in the tests where the final pH became more acidic, the highest EPS productions occurred, confirming that acidity is an important condition. The combination of $(NH_4)_2SO_4$ and $NaNO_3$ led to a sharp drop in pH (pH 2.0), which favored EPS production (Table 1). Some authors describe that the consumption of ammonium salts by the yeast results in the release of protons, causing acidification of the medium [39, 49]. This helps to explain the results obtained with *C. laurentii* SD7 since one of the nitrogen sources was $(NH_4)_2SO_4$. In addition, molasses presents amino acids and proteins that may have increased the presence of available nitrogen and, consequently, the consumption by the yeast increased, leading to the accentuation of this condition [30, 31].

3.3. Optimization of EPS production by response surface methodology

Sugar cane molasses, $(NH_4)_2SO_4$ and $NaNO_3$ were analyzed to obtain their optimum levels to maximize the EPS production by *C. laurentii* SD7 with an CCD 2^3 (Table 2). The highest EPS productions occurred in assay 2, 4, 8, 10, and 13, presented a production between 7.0 g L^{-1} and 11.0 g L^{-1}.

Table 2: A Central Composite Design 2^3 with coded and real variables, for EPS production by Cryptococcus laurentii SD7 in sugar- cane molasses, $NaNO_3$ and $(NH_4)_2SO_4$.

Assay	Coded variables			Real variables			Response
				Molasses	$NaNO_3$	$(NH_4)_2SO_4$	EPS
	X_1	X_2	X_3	(%)	(%)	(%)	(g L^{-1})
1	-1	-1	-1	2.6	0.3	0.3	2.61
2	1	-1	-1	7.4	0.3	0.3	8.46
3	-1	1	-1	2.6	0.7	0.3	2.24
4	1	1	-1	7.4	0.7	0.3	7.60
5	-1	-1	1	2.6	0.3	0.7	3.24
6	1	-1	1	7.4	0.3	0.7	5.35
7	-1	1	1	2.6	0.7	0.7	3.57
8	1	1	1	7.4	0.7	0.7	7.12
9	-1.68	0	0	1.0	0.5	0.5	4.52
10	1.68	0	0	9.0	0.5	0.5	7.60
11	0	-1.68	0	5.0	0.2	0.5	4.02
12	0	1.68	0	5.0	0.8	0.5	4.42
13	0	0	-1.68	5.0	0.5	0.2	11.0
14	0	0	1.68	5.0	0.5	0.8	4.61
15	0	0	0	5.0	0.5	0.5	4.56
16	0	0	0	5.0	0.5	0.5	3.99
17	0	0	0	5.0	0.5	0.5	4.48

The three-dimensional graph shows that the EPS concentration increased with the increasing molasses concentration (Figure 2 A and B). For $NaNO_3$, on the other hand, the concentration range studied did not present significant effects, indicating that its variation did not affect the production of EPS ($p \geq 0.1$) (Table 3); thus, making it possible to work with the lowest concentration (Figure 2 A and B). Regarding $(NH_4)_2SO_4$, the maximum production of EPS occurred at the lowest concentration (Figure 2C).

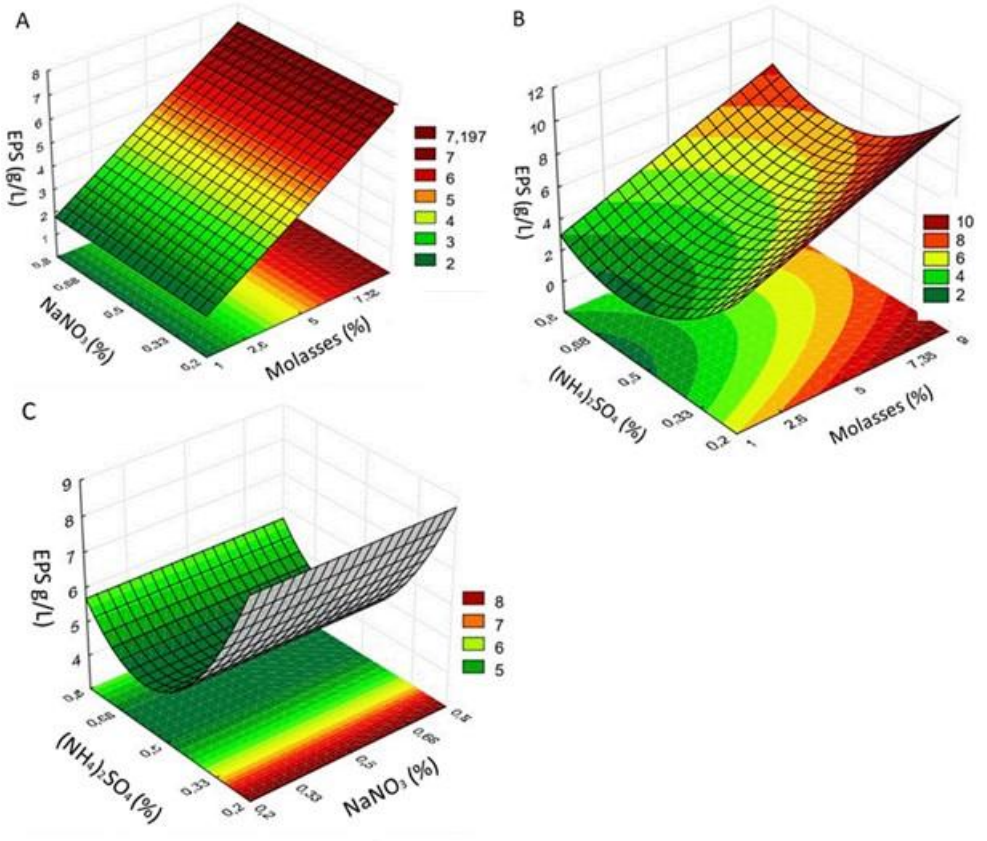

Figure 2. Response surface for EPS production by Cryptococcus laurentii SD7. A. sugar cane molasses x $NaNO_3$; B. sugar cane molasses x $(NH_4)_2SO_4$; C. $NaNO_3$ x $(NH_4)_2SO_4$

The analysis of the regression coefficients showed that the only factors that affected the production of EPS were sugar cane molasses and $(NH_4)_2SO_4$ considering a 10% confidence level ($p < 0.1$) (Table 3).

Table 3: Regression analysis of EPS production by Cryptococcus laurentii SD7 using sugar cane molasses, $(NH_4)_2SO_4$ and $NaNO_3$.

Factors	Regression coefficient	Standard error	t (7)	*p*-value
Average	4.41	0.94	4.67	0.002268
Molasses (*L)	1.61	0.44	3.64	0.008276
Molasses (**Q)	0.36	0.48	0.74	0.482997
$NaNO_3$ (L)	0.11	0.44	0.25	0.807386
$NaNO_3$ (Q)	- 0.28	0.48	- 0.59	0.571954
$(NH_4)_2SO_4$ (L)	- 0.90	0.44	- 2.04	0.080691
$(NH_4)_2SO_4$ (Q)	0.97	0.48	2.00	0.085410
Molasses x $NaNO_3$	0.11	0.57	0.20	0.844990
Molasses x $(NH_4)_2SO_4$	- 0.69	0.57	- 1.19	0.270716
$NaNO_3$ x $(NH_4)_2SO_4$	0.41	0.57	0.72	0.494327

*L=linear; **Q=quadratic; R^2= 0.78; 10% confidence level.

The value of F_{calc} of 14.83 indicated that the results were highly significant, and the correlation between predicted and observed values was very good (Table 4). The results fit the model well, and the values of the regression coefficients can be used to generate the following model equation as follow:

$$Y = 4.41 + 1.61X_1 + 0.11X_2 – 0.90X_3 + 0.36X_1^2 – 0.28\ X_2^2 + 0.97\ X_3^2 + 0.11X_1X_2 – 0.69X_1X_3 + 0.41X_2X_3$$

Table 4: Analysis of variance (ANOVA) for EPS production by Cryptococcus laurentii SD7 using sugarcane molasses, $NaNO_3$ and $(NH_4)_2SO_4$.

Factors	Sum of squares	Freedom degree	Medium square	Fc*
Regression	58.75	2	29.37	14.83
Residue	27.7	14	1.98	
Lack of fit	27.51	11		
Pure error	0.19	2		
Total	86.45	16		

*Fc=F calculated; F_{tab}: 2.72; 10% confidence level.

The optimization of EPS production by *C. laurentii* SD7 using CCD increased the production by about 30%, reaching 11.0 g L^{-1} after 96 h. Furthermore, the combination of inorganic salts was promising for *C. laurentii* SD7. These results were almost three times higher EPS production compared with the combination of sugar cane molasses and corn steep liquor as a nitrogen source, 4 g L^{-1} after 96 h, which was reported by Silva et al. (2022) [19].

The concentration of the carbon source is a determining factor in EPS production because it is directly proportional to the production of EPS [22, 40]. However, some authors have shown that very high concentrations of carbon can negatively affect EPS production [27, 42, 43, 50, 51]. This phenomenon is known as catabolic repression, a condition in which microbial growth is favoured and product biosynthesis usually decrease [52]. Furthermore, with the increase in EPS synthesis, the viscosity of the medium increases, making it difficult to distribute oxygen and nutrients to the cells, which decreases productivity [49].

Studies show that the use of $(NH_4)_2SO_4$ is efficient for the production of EPS [35, 51, 53-55]. Some authors report that the C:N ratio can affect EPS production because while high values of carbon source are required, high values of nitrogen can inhibit EPS production due to the repression of the metabolic pathway. Pavlova et al. (2004) [32] reported that concentrations between 0.2% and 0.3% of inorganic salts are ideal for EPS production. The results obtained by *C. laurentii* SD7 corroborate the reports in the literature, showing that the lowest concentrations of $(NH_4)_2SO_4$ and $NaNO_3$ were the best for the production of EPS production.

Obtaining good results with inorganic salts as a source of nitrogen is interesting and promising from an economic point of view, as they are much less expensive than complex substrates such as yeast extract and peptone. When combined with renewable and low-cost sources like agro-industrial by-products, such as sugarcane molasses, this approach becomes even more economically advantageous. Additionally, it offers environmental benefits by adding value to these wastes, contributing to their recycling, and supporting the circular economy.

4. CONCLUSIONS

Using *Cryptococcus laurentii* SD7, a high exopolysaccharide (EPS) synthesis was observed. The combination of sugarcane molasses with inorganic nitrogen sources proved efficient for both EPS and microbial biomass production, yielding good results with process stability. This strategy enables a reduction in production costs by replacing conventional, more expensive media components. Additionally, the EPS obtained exhibited hard and resistant characteristics, indicating potential for application in the development of biodegradable materials as an alternative to conventional plastics derived from fossil sources.

5. ACKNOWLEDGMENT

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