BIOSURFACTANT PRODUCTION BY FUNGI ASSOCIATED WITH CASSAVA STEMS AND LEAVES AIMED AT INDUSTRIAL APPLICATION

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ABSTRACT

The production of compounds with the potential to act as biosurfactants by fungi obtained from cassava stems and leaves (12 fungi in total) was evaluated. The analysis from each fungus showed a higher production of these compounds by the CA10 fungus (Vouchered mycorrhizae). This fungus was submitted to growth in a specific liquid culture medium, varying the time, temperature, and pH to increase biosurfactant production. The analysis of the results referring to the reduction of surface tension showed that the ideal condition for the fungus growth was a fermentation process with a temperature of 29ºC, pH 7.6, and a time of 15 days. This condition promotes a reduction of surface tension of 51.97% compared with water.

Keywords: Fungus. Biosurfactant. Surface tension.

1 INTRODUCTION

Synthetic surfactants are used in almost all segments of the industry due to their wide versatility, as they are products that harmoniously transition from detergents used for home or industrial cleaning through personal care formulations, standardizing formulations in agricultural applications, to applications in oil fields for extraction, among others.[1] However, the environmental impacts related to the use of surfactants have attracted attention due to their toxicity and low biodegradability, leading to severe environmental problems.[2] Therefore, research has been carried out in the search for sustainable substitutes, with biosurfactants being a promising alternative to synthetic surfactants. This characteristic is because these compounds are mainly biodegradable, present lower toxicity, and are more stable products in extreme temperature, salinity, and pH compared to their synthetic equivalents.[3]

In this context, the global industries have been investing in this market, focused mainly on glycolipids, lipopeptides, lipoproteins, phospholipids, and fatty acids, among others. However, some limitations and challenges related to the application and production of these materials to replace synthetic surfactants are observed, such as meeting physical-chemical properties and production yield, the high cost of raw materials and the production process, and the economic challenge regarding commercialization. In addition to these limitations, the search for expansion and diversification of products to be offered on the market, whether through different sources of microorganisms, as proposed in this work, or through the implementation of improvements in the production process, as well as the gain of scale, stimulating continued research for new biosurfactant sources.[4] Therefore, given the enormous potential for different possibilities for industrial applications of biosurfactants and taking into account that the bioproducts most used in this field come from *Pseudomonas and Bacillus*, the importance of developing the present study, which aims to evaluate the production of compounds that have potential for application as biosurfactants, through fungi associated with cassava stems and leaves, aiming at the introduction of new microorganisms with potential for producing these compounds.

2 MATERIAL & METHODS

2.1. Biosurfactant Production

Fungi were isolated from cassava stems and leaves and maintained at 4ºC on potato dextrose agar (PDA). Afterward, the fungi underwent three consecutive growths in a solid PDA medium previously sterilized in an autoclave to activate the metabolic system. After this process, gentamicin sulfate was added as an antibiotic agent. Fungal growth was carried out for 7 days at 23ºC in a Panasonic air-conditioned chamber. After the third propagation, the fungi were subjected to growth in a liquid medium to optimize the conditions for biosurfactant production. All fungi were subjected to growth in a liquid medium composed of peptone, glucose, yeast extract, ammonium sulfate, and olive oil. The culture medium was previously sterilized in an autoclave, and after this process, gentamicin sulfate was added as an antibiotic. The growth time was 7 days, temperature 30ºC, pH 6, and shaker rotation of 180 rpm. Afterward, the mycelia were separated by vacuum filtration, followed by centrifugation at 6000 rpm, and the extracts obtained were subjected to characterization via evaluation of the surface tension reduction. The potential to act as a biosurfactant was compared with the standard sodium dodecyl sulfate solution.

2.2.2. Biosurfactant Production Optimization

After selecting the fungus with the most significant potential for biosurfactant production (Vouchered mycorrhizae), it was again subjected to growth in a liquid medium. The time (2.3 to 15.7 days), temperature (18.9 to 39.1 ºC), and pH (2.6 to 9.4) are modified to optimize the production of this class of compounds. The pH of each medium was adjusted by adding NaOH or HCl solution,

respectively. The rotation used during each growth was 180 rpm. After fermentation, each system was filtered and then centrifuged at 6000 rpm for 30 min, with the filtrate used to evaluate biosurfactant production. The potential to act as a biosurfactant was compared with the standard sodium dodecyl sulfate (SDS). Central rotational composite planning with two center points was used to evaluate the efficiency of the fungus's production of biosurfactants in different cultivation conditions, with surface tension data considered when determining the best growth condition of the fungus in a liquid medium. The results were evaluated through the analysis of the contour surfaces, as well as using the desirability function and evaluation of the variables that most influenced the production of biosurfactants. Data were analyzed using the STATISTICA 14.0 program.

3 RESULTS & DISCUSSION

12 fungi isolated from stems and leaves of cassava (*Cladosporium xanthochromaticum* – LV2, *Xylaria* sp – LV3, *Microdochium lycopodinum* - ST1, *Alternaria* sp.- ST5, *Diaporthe endophytica* – ST9, *Vouchered mycorrhizae* – ST10, Phanerochaete australis – ST11, *Diaporthe caatingaensis* – ST12, *Stenocarpella maydis* – ST13, *Annulohypoxylon stygium* – ST14, *Sordariomycetes* sp. – ST15, *Phanerochaetaceae* sp.- ST17) were evaluated regarding their capacity to produce compounds with the potential to act as biosurfactants. Each fungus was initially subjected to three consecutive subcultures in a PDA medium to activate the enzymatic system and promote the production of compounds with the potential to act as biosurfactants. After the third recirculation, they were subjected to growth in a liquid medium. Once the process was complete, they were evaluated concerning the reduction in surface tension and the value of the emulsification index (Figure 1).

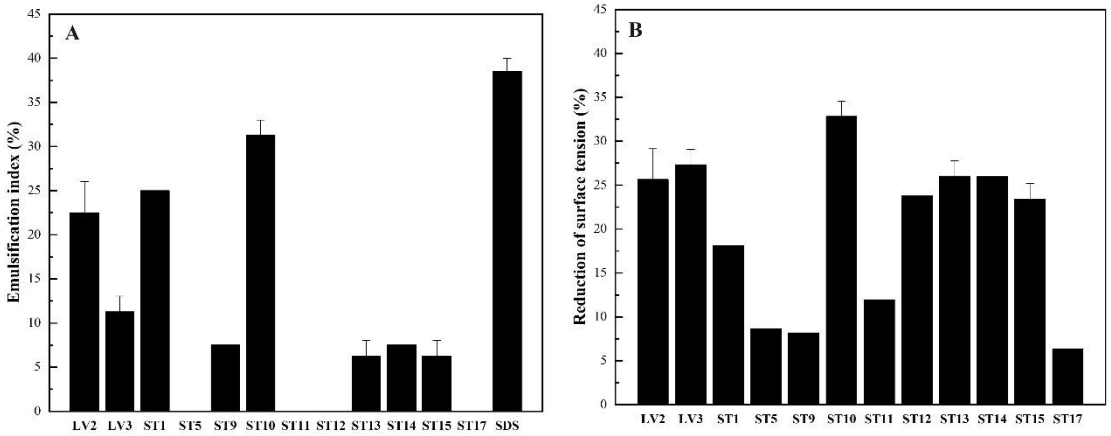

Figure 1 Data obtained for the initial extracts: A) Emulsification index; B) Reduction of surface tension

It is possible to verify a high production of biosurfactants by the ST10 fungus, which led to emulsification levels close to half the value of the SDS standard, as well as reductions in surface tension more significant than 40%, which shows the efficiency of the extract produced since these values were obtained without carrying out purifications. Significant results were observed for extracts LV2 and ST1, which also showed good reductions in surface tension and significantly high emulsification indices. After verifying the production efficiency of compounds capable of acting as biosurfactants by the fungus ST10, the growth condition was optimized, aiming to maximize the production of biosurfactants. In this way, the pH of the culture medium was modified, as well as the growth time and temperatures (Table 1). This assessment is since changes occur in the production of compounds from microorganisms due to the variation in growth conditions and, specifically, due to the more outstanding production of this class of compounds in the exponential phase of growth of the species.

Analyzing the surface tension reduction data tests, using time above 9 days favored the production of compounds that reduced surface tension and temperatures close to 29 ºC and a slightly acidic pH. Therefore, considering the data obtained, the influence of each input variable (pH, time, and temperature) on the production of biosurfactants was evaluated, and the ideal condition for carrying out the fermentation process was determined, aiming to maximize the production of this class of compounds by the fungus. Analyzing the results using the Pareto chart, in which the effect of each variable is standardized and the interaction evaluated with a confidence level of 95%, it appears that the variable that most influenced the production of the desired compounds was the temperature (quadratic term) (Figure 2). This data can be explained by the reduction in the growth of the fungus at high temperatures due to its low resistance and destruction of the mycelia. Although not considered in the analysis as the most relevant factor, it is verified that the growth time (linear mode) also proved to be an essential variable since the fungus needs sufficient time for growth and secretion of the compounds of interest. pH (6–9) was not a fundamental parameter in the fungus's growth and biosurfactants' production.

Table 1 Growth conditions used and data surface tension reduction

Coded variables			Uncoded variables			Output variable
T (ºC)	pH	Time (day)	T (ºC)	pH	Time (day)	Surface tension reduction (%)
0	0	+1.69	29	6	15	25.32
-1.69	0	0	18.9	6	9	1.34
0	-1.69	0	29	2.64	9	12.66
0	0	-1.69	29	6	2.3	18.50
0	+1.69	0	29	9	9	15.86
-1	+1	-1	23	8	5	6.54
-1	-1	-1	23	4	5	14.90
+1	+1	-1	35	8	5	1.99
+1.69	0	0	39.1	6	9	10.43
+1	+1	+1	35	8	13	18.11
0	0	0	29	6	9	17.67
0	0	0	29	6	9	18.59
+1	-1	-1	35	4	5	3.62
+1	-1	+1	35	4	13	7.19
-1	+1	+1	23	8	13	14.75
-1	-1	+1	23	4	13	14.29
Culture medium						48
SDS						33
Water						70

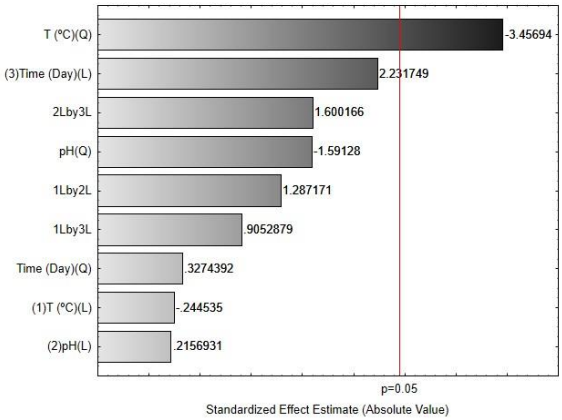

Figure 2 Assessment of the influence of each input variable on biosurfactant production

Through the analysis of the contour surfaces (Figure 3), it was also possible to determine that temperatures between 27°C and 35ºC, slightly basic pH, and times longer than 10 days favor the production of compounds with the potential to act as biosurfactants. It has also been found that acidic pHs are detrimental to the production of these compounds, and they are very low in time, which is not enough to activate the fungus' metabolism. Using the desirability function, it was possible to determine the ideal condition for fungus growth and production of compounds with the potential to act as a biosurfactant (Figure 4). This condition used a pH equal to 7.6, a temperature of 29ºC, and a growth time of 15 days.

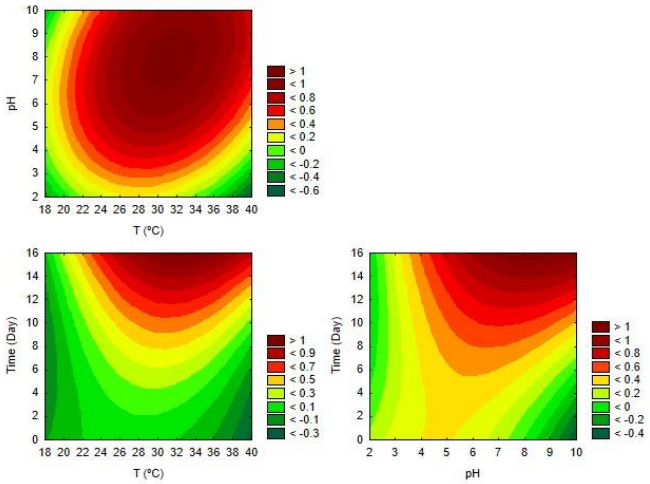

Figure 3 Contour surfaces obtained during the optimization of biosurfactant production by ST10

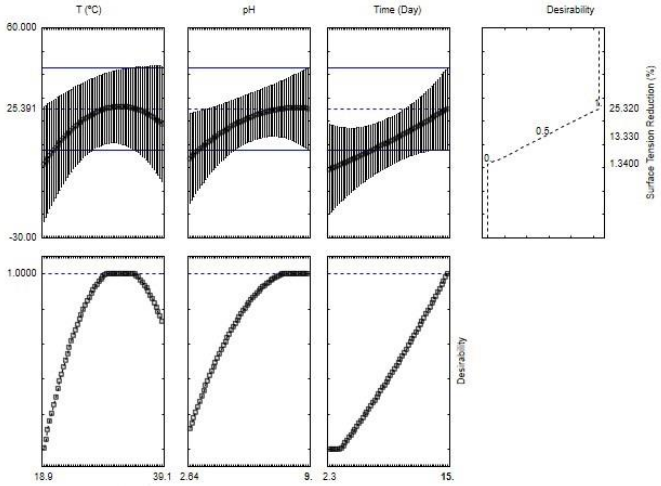

Figure 4 Optimal fermentation condition for biosurfactant production by ST10

4 CONCLUSION

During the development of the present work, it was possible to verify the production of biosurfactants by fungi associated with cassava stems and leaves and evaluate the influence of the parameters temperature, pH, and time after characterizing the material via the determination of surface tension, the highest production of these compounds by the fungus *Vouchered mycorrhizae* (ST10). Therefore, the possibility of having alternatives to the production of biosurfactants is verified, replacing bacteria with fungi originating from cassava stems and leaves for mapping the application areas.

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ACKNOWLEDGEMENTS

This work was supported by grants #2015/19273-2 from the São Paulo Research Foundation (FAPESP).