I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIOPROCESS ENGINEERING

GROWTH EVALUATION AND BIOSURFACTANT PRODUCTION BY *Candida mogii* IN A MEDIUM CONTAINING LICURI (*Syagrus coronata*) OIL

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ABSTRACT

Biosurfactants are biodegradable and low-toxicity compounds derived from microorganisms. Due to the high costs associated with production, new strains and substitute sources of production are often explored. This study investigates the potential of *Candida mogii* as a biosurfactant producer using licuri oil as an alternative substrate. The methodology involved culturing *C. mogii* in a medium containing licuri oil and glucose. The cultures were incubated, and samples were collected every 24 hours to determine the production parameters over the time. Biosurfactant production was indirectly measured by determining the surface tension in the metabolic liquid supernatant. The results showed that even under non-optimized conditions, *C. mogii* was able to reduce surface tension of the water from 71.04 ± 0.02 to 36.60 ± 0.14 $mN.m^{-1}$ after 168 hours of fermentation. Biosurfactant production was observed to commence during the logarithmic phase, progressing through the stationary phase until reaching its peak at the onset of the decline phase, with a yield of 2.54 ± 0.29 $g.L^{-1}$. In conclusion, *C. mogii* shows potential for biosurfactant production using licuri oil as a carbon source. However, further research is necessary to optimize, characterize, and explore the potential applications of this bioproduct.

Keywords: Yeast. Fermentation. Ouricuri oil.

1 INTRODUCTION

The global biosurfactants market is projected to reach $6.3 billion by 2026. This growth is primarily driven by the increasing demand in the sanitation sector, which can be attributed to the SARS-CoV-2 pandemic and the resulting hygiene-related behavioral changes.[1] Biosurfactants (BS) are compounds derived from microorganisms that offer several benefits, including biodegradability, low toxicity, and cost-effective production from renewable materials. They are used in many applications, such as bioremediation, oil recovery, and serving as antimicrobial agents.[2]

BS exhibit diverse chemical structures and properties and can be obtained from various sources, including bacteria, fungi, and yeast.[3] Despite concerns about their pathogenicity, *Candida* species are recognized as potent surfactant producers. They can develop on both miscible and immiscible substrates, offering glycolipids, polymeric surfactants, and fatty acids.[4]

To optimize biosurfactant production, it is crucial to explore novel carbon sources. Vegetable oils show promise as substrates for biomolecule synthesis beyond surfactant properties. Licuri (*Syagrus coronata*) oil, rich in medium-chain fatty acids, is particularly an attractive candidate for lipid-derived biomolecule production.[5,6] In this context, the present study evaluates the kinetics of surfactant production by *Candida mogii* using Licuri oil as an alternative carbon source.

2 MATERIAL & METHODS

The yeast strain used as a biosurfactant producer was *Candida mogii* (UFPEDA 3968), which is deposited in the Culture Collection of the Mycology Department of the Federal University of Pernambuco. The cultures were kept at 5ºC in assay tubes containing Yeast Mold Agar (YMA) medium, which consists of the following components ($g.L^{-1}$): yeast extract (3), malt extract (3), D-glucose (10), tryptone (5), and agar (2). Next, the yeast was transferred to Erlenmeyer flasks containing 50 mL of Yeast Mold Broth (YMB) medium, which has the same composition as YMA, except for the agar. The flasks were incubated under shaking at 150 rpm at 28ºC for 24 hours in an incubator shaker model C25KC (New Brunswick Scientific, Edison, NJ, USA).[7]

The medium used was composed of the following components ($g.L^{-1}$): Licuri oil (30), glucose (60), NH_4NO_3 (2), KH_2PO_4 (0.1), $MgSO_4 \cdot 7H_2O$ (5g), $FeCl_3$ (0.1), and NaCl (0.1). The yeast was maintained in this medium under the following conditions: 216 hours, 28ºC, 200 rpm, with 1% of the inoculum of 10^7 CFU/mL, counted in a Neubauer chamber. The experiments were conducted in triplicate, and samples were collected from the medium every 24 hours to determine the optimal production time, considering the given specifications. For each sample, the pH was measured using MS Tecnopon digital potentiometer, the biomass was collected from the culture through centrifugation (Rotina 420R, Hettich Zentrifugen, Tuttlingen, Germany) at 4500 rpm for 10 minutes. The sediment was then washed with a sodium chloride solution (10 $w.v^{-1}$%) and dried in an oven at 70ºC for 24 hours. The dry cell weight (DCW) was measured by weighing the dried sediment. To calculate the concentration of biomass (C_X, $g.L^{-1}$), the DCW was divided by the volume of culture.[8]

The biosurfactant production was indirectly measured by determining the surface tension (ST) in the metabolic liquid supernatant using the NUOY ring on a KSV Sigma 700 tensiometer from Finland. The consumption of glucose was measured using

spectrophotometry (Model GT7220, Global Analyzer, Brazil) at 540 nm after reacting with 3.5 dinitro-salicylic acid. Liquid-liquid extraction with hexane was employed to evaluate the consumption of licuri oil in the medium over time. The biosurfactant was obtained via liquid-liquid extraction using ethyl acetate. The extraction process involved mixing the solvent and metabolic liquid in a 1:4 ratio, repeating the process thrice, and centrifuging the solvent part. The organic phase was transferred to a separation funnel, washed with a saturated NaCl solution, dried, filtered, and then evaporated to obtain the isolated biosurfactant, which was quantified using gravimetry. Measurements were reported as the mean value ± error.[7]

3 RESULTS & DISCUSSION

Figure 1 shows the evaluation of biomass concentration, yield, reduction of surface tension, and substrate consumption (hydrophilic and hydrophobic) by *Candida mogii* UFPEDA 3968 yeast during the pre-established fermentation period.

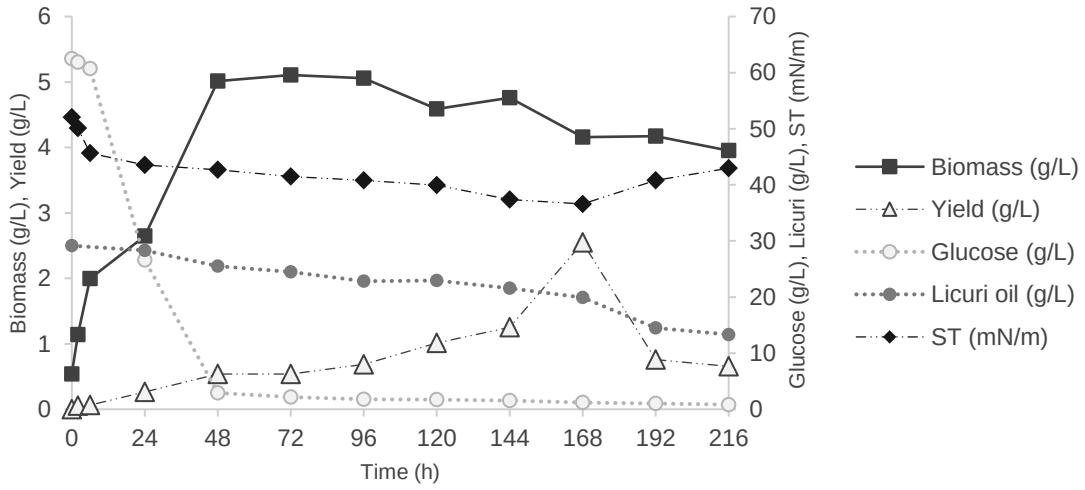

Figure 1 Temporal changes of biomass, yield, glucose, licuri oil and surface tension during cultivation of *C. mogii* in mineral medium.

The representative biomass curve reaches its maximum point (5.13 ± 0.08 $g.L^{-1}$) during the first 72 hours of fermentation while glucose is still present in the medium. The low complexity of the hydrophilic source increases the availability of carbon in the medium, favoring its primary use by cells as an energy source. As this source becomes depleted, there is a reduction in cell growth, entering the stationary phase, where the carbon source must be obtained from the fatty acid chains present in licuri oil. The hydrophobic nature of oils results in prolonged resistance against biodegradation due to their low water solubility. This phenomenon increases their adsorption to cell surfaces, reducing their availability to degrading microorganisms.[8] However, the increase in yield, continuous reduction in surface tension and oil quantity, reveals its consumption by yeast over time, demonstrating that it can be used as a carbon source in the BS production.

The balance between hydrophobic and hydrophilic sources in the cultivation medium stands as one of the pivotal factors influencing the quantity and characteristics of the produced biosurfactants (BS). In the present study, a ratio of 60:30 was employed, yielding a maximum efficiency of 2.54 ± 0.29 $g.L^{-1}$ within the 168-hour interval, thereby generating a productivity of 15.71 ± 0.35 $mg.L^{-1}.h^{-1}$.Previously, alternative ratios of 10:10 (glucose : soap stock), resulting in a production rate of 49.96 $mg.L^{-1}.h^{-1}$; 100:100 (glucose : sunflower oil refinery waste), yielding a sophorolipid production rate of 216 $mg.L^{-1}.h^{-1}$; and 100:30 (glucose : rapeseed oil), obtaining a rate of 1.64 $g.L^{-1}.h^{-1}$ were reported as optimal carbon source compositions rate for biosurfactant production in their respective conditions.[9,10,11]

While there is no register on biosurfactant production using *C. mogii*, *Candida* spp. has been shown to have a strong ability to reduce surface tension. *C. bombicola*, for example, in optimized media was able to achieve a water ST reduction from 72 to 29 $mN.m^{-1}$.[7] Similarly, *C. tropicalis* produced biosurfactant that reduced ST to 25.8 $mN.m^{-1}$ through solid-state fermentation, a result comparable to that obtained by bacteria.[12] In this study, the biosurfactant produced by *C. mogii* presented a reduction of surface tension from 71.04 ± 0.02 to 36.60 ± 0.14 $mN.m^{-1}$ after 168 hours of cultivation in non-optimized medium.

A biosurfactant that functions efficiently is capable of significantly lowering the surface tension of water, by reducing it from 72 to 35 $mN.m^{-1}$, the yeast strain used in this research exhibits potential for producing biosurfactants. Given that the optimization of the bioprocess is pivotal for biosurfactant production, it significantly impacts both the total production yield and the ability to reduce surface tension.[13]

In this study, biosurfactant production was observed to commence during the logarithmic phase, progressing through the stationary phase until reaching its peak at the onset of the decline phase. This finding is consistent with studies involving *C. catenulata* and *C. tropicalis*, which detected sophorolipid production as early as the log phase, although these same studies reported higher yields and surface tension reduction during the stationary phase.[8,14] However, when using a different strain of *C.*

tropicalis, researchers reported higher yields and greater surface tension reduction even during the log phase. Since yeast strains exhibit diverse mechanisms underlying sophorolipid production, the relationship varies accordingly. This suggests that biosurfactant production and surface tension reduction are not necessarily associated with cell growth.[15]

The pH profile evaluated throughout the fermentation process (data not shown) exhibited a variation from 5.06 ± 0.02 to 2.9 ± 0.02 within the initial 24 hours, culminating at 2.58 ± 0.02 by the conclusion of the fermentation period.

4 CONCLUSION

This study aimed to investigate the potential of *Candida mogii* as a novel surfactant-producing species from the Candida genus, using a predefined mineral medium. The results of this study indirectly demonstrated the ability of the yeast strain to grow and produce biosurfactants when using licuri oil as an alternative carbon source. Remarkably, even under non-optimized conditions, *C. mogii* was able to reduce surface tension to approximately 35 $mN.m^{-1}$ with a yield of 2.54 $g.L^{-1}$ after 168 hours of fermentation. This makes it an attractive candidate for biosurfactant production. Further research is necessary to optimize, characterize and explore the potential applications of this bioproduct.

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ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors express their gratitude to the Bioprocess Laboratory of the Antibiotics Department at the Federal University of Pernambuco, Science and Technology Center of the Catholic University of Pernambuco (UNICAP) and the Cooperativa de Produção da Região do Piemonte da Diamantina (COOPES) for providing the fixed oil of *S. coronata*.