Candida mogii: A PROMISSING YEAST FOR BIOSURFACTANT PRODUCTION

Peterson F. F. da Silva[1]*, Jorge L. S. Sonego[2] & Jenyffer M. C. Guerra[1]

[1] *Chemical Engineering Graduate Program, Department of Chemical Engineering, Federal University of Pernambuco, Recife, PE, Brazil.*
[2] *Biotechnology Graduate Program, Department of Antibiotics, Federal University of Pernambuco, Recife, Brazil.*
** Corresponding author's email address: peterson.fsilva@ufpe.br.*

ABSTRACT

Biosurfactants offer eco-friendly, low-toxicity alternatives to petrochemical-based synthetic surfactants. Despite their advantages, production cost challenges widespread adoption. This study aims to explore *Candida mogii* surfactant production, evaluating its potential as a bioemulsifier. The yeast strain was cultured in mineral medium supplemented with glucose and Licuri oil for 168h at 28°C, 200 rpm. Biosurfactant presence was indirectly measured via surface tension analysis. Emulsion formation ability was assessed using various hydrocarbon sources and evaluated over 30 days. Additionally, stability tests were conducted at different temperatures, NaCl concentrations, and pH levels. Results shows that *C. mogii* can reduced surface tension from 71.04 to 36.6 mN/m, indicating potential biosurfactant production. Emulsification tests showed excellent affinity with hydrocarbons (E_{24} above 75%). Stability tests revealed promising results under extreme conditions of pH, temperature, and salinity, suggesting versatile applications. Further research is required to enhance production and explore applications.

Keywords: Yeast. Bioemulsifier. Stability.

1 INTRODUCTION

Biosurfactants (BS) represent a class of amphiphilic molecules synthesized by microorganisms, manifesting as active surface agents. Unlike synthetic surfactants, which are made from petrochemicals, BS are eco-friendly and have low toxicity. They are also adaptable for use over a wide range of temperature, salinity, and pH.[1]

However, despite these advantages, the cost of the production process remains a challenge for their widespread adoption. Consequently, there is a growing need for studies aimed at identifying optimal producer strains and cultivating conditions (such as substrate composition, temperature, agitation, and aeration) to maximize production yields.[2]

To evaluate the quality of BS production, preliminary tests can be applied to assess their ability to reduce surface and interfacial tension. While not all biomolecules are able to fulfill this role, they still serve as effective emulsifying agents, contributing to the formation and stability of emulsions. In this context, our work aims to explore BS production using *Candida mogii* yeast, through an evaluation of its ability to reduce surface tension and act as a bioemulsifier (BE).

2 MATERIAL & METHODS

The yeast strain for biosurfactant production was *Candida mogii* UFPEDA 3968, maintained in the Culture Collection of the Mycology Department at the Federal University of Pernambuco. Cultures were stored at 5°C in assay tubes containing Yeast Mold Agar (YMA) medium, composed of yeast extract (3 g/L), malt extract (3 g/L), D-glucose (10 g/L), tryptone (5 g/L), and agar (2 g/L). Subsequently, the yeast was inoculated into Erlenmeyer flasks containing 100 mL of Yeast Mold Broth (YMB) medium, with the same composition as YMA but excluding agar. The flasks were incubated at 28°C and 200 rpm for 24 hours in a C25KC incubator shaker (New Brunswick Scientific, Edison, NJ, USA).The medium was composed of the following components: licuri *(Syagrus coronata)* oil (30 g/L), glucose (60 g/L), NH_4NO_3 (2 g/L), KH_2PO_4 (0.1 g/L), $MgSO_4 \cdot 7H2O$ (5 g/L), $FeCl_3$ (0.1 g/L), and NaCl (0.1 g/L). The yeast was cultured in this medium under the following conditions: 168 hours, 28°C, 200 rpm agitation, with an initial inoculum of 1% containing 10^7 colony-forming units (CFU)/mL, quantified using a Neubauer chamber. After 168 hours, the culture medium was immediately subjected to centrifugation (Routine 420R, Hettich Zentrifugen, Tuttlingen, Germany) at 4500 rpm for 10 minutes. From the supernatant, cell-free metabolic liquid, the presence of biosurfactant could be indirectly measured by determining the surface tension in the medium using the NUOY ring on a KSV Sigma 700 tensiometer from Finland.[3]

The ability to form emulsions was evaluated from an adaptation of the method developed by Cooper and Goldberg[4], where the metabolic liquid was tested against various hydrocarbon sources (engine oil, burnt engine oil, canola oil, and sunflower oil) in a 1:1 ratio in test tubes. Each tube was subjected to vortex agitation for 2 minutes and after 24 hours, the emulsification index (E24) could be determined using Equation 1.

$$E_{24} = \frac{h_e}{H} \times 100 \quad (1)$$

Where h_e denotes the height of the emulsion column and H represents the total height of the column formed by the merging of the two liquids, the outcome is expressed as a percentage.

To evaluate the biosurfactant's stability in emulsion formation, the formed emulsion was kept for 30 days to assess its behavior over time. Additionally, the metabolic liquid was exposed to different temperatures (0, 60, and 121°C) for 30 minutes, varying NaCl concentrations (1, 5, and 10%), and different pH levels (1, 7, and 12). Subsequently, emulsifying activities were determined following the previously described methodology. All analyses were conducted in triplicate.

3 RESULTS & DISCUSSION

After 168 hours of fermentation by *C. mogii* in a mineral medium containing 6% glucose and 3% licuri oil, the metabolic liquid obtained showed a remarkable reduction in surface tension from 71.04 to 36.6 mN/m. This indicates the potential production of biosurfactant by the yeast, as measuring surface tension is a widely accepted method for detecting surface-active biomolecules in the culture medium.[5] An effective biosurfactant is one that can reduce surface tension to values below 35 mN/m.[6] Optimized processes generally yield better results in terms of tension reduction.[3] Therefore, the biosurfactant produced by the studied microorganism in a non-optimal medium, reaching values close to the acceptable limit, can be considered promising. The pH at the beginning of fermentation was 5.06, dropping to 2.57 by the end of the process.

The emulsification index determines how effectively the biosurfactant functions as a bioemulsifier. While emulsification and dispersion additives don't necessarily lower the surface tension of water or hydrocarbons, they do assist in reducing the surface energy between phases.[2] To be considered a good emulsifying agent, the ability to form stable emulsions must remain above 50% for at least 24 hours.[7] The results of the emulsification tests demonstrated an excellent affinity between the metabolic liquid and the 4 tested hydrocarbons, with the highest percentage obtained in the emulsification of engine oil (81.48%), followed by licuri oil (81.25%), canola oil (80.77%), and burnt engine oil (75%), as shown in Figure 1. The stability of all evaluated emulsions also maintained satisfactory values even after 30 days, with the best presented by canola oil, with a reduction of 7.69%.

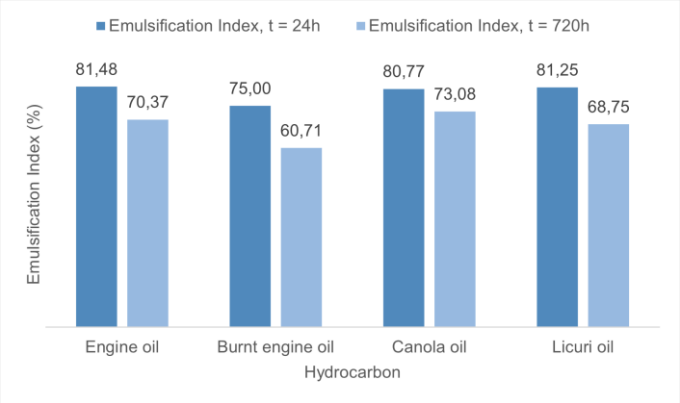

Figure 1 Emulsification index observed after 24 hours (E24) and 720 hours (30 days) of storage.

Araujo et al. obtained similar results with BE4CK produced by *C. krusei* M4CK, capable of satisfactorily emulsifying hydrocarbons (above 50%), but showed a reduction in surface tension from 72 mN/m to 44 mN/m.[8] Similarly, Bhaumik et al. produced a biomolecule from *Meyerozyma caribbica* that could only reduce surface tension to 54 mN/m but exhibited emulsification indices above 70%.[9] A good Bioemulsifier/Biosurfactant must remain stable across different temperature, pH, and salinity conditions to be viable in various industries.[3]

Figure 2 shows the stability of the biosurfactant produced by *C. mogii* evaluated from the cell-free metabolic liquid under extreme conditions of pH, temperature, and salinity. pH can change the molecular charge of the surfactant, consequently affecting the molecule's performance regarding its orientation at the interface.[10] In tests involving engine oil and burnt engine oil, the E_{24} maintained values above 80%, remaining almost unaffected within the tested pH range (1, 7, and 12). On the other hand, canola oil showed a reduction of more than 50 percentage points in the most extreme pH ranges (pH 1 and 12), while Licuri oil experienced about a 30% reduction in the acidic range, remaining stable in the others compared to the primarily conducted emulsification activity.

Regarding the effects of temperature, significant changes were not observed under the tested conditions (0°C, 60°C, and 121°C) when compared to the emulsification activity observed initially, for all hydrocarbons. Such results demonstrate that the biomolecule is thermostable. The decline in emulsifying activity could be attributed to the denaturation of the protein component of the bioemulsifier when subjected to heat treatment. This characteristic relies on the chemical structure of the molecule, as numerous emulsifiers remain stable across wide temperature ranges or exhibit only slight variations at elevated temperatures.[9]

When compared to the initially obtained E_{24} in this study, a 1% NaCl salinity showed no significant alteration, remaining around 80% for all tested hydrocarbons. As the sodium chloride concentration increases (5% and 10%), all hydrophobic media experienced a reduction in their emulsification activity but remained above 60%. These results are satisfactory as they indicate potential use in a range of applications, including motor oil recovery from seawater (which typically contains around 2% NaCl salinity).

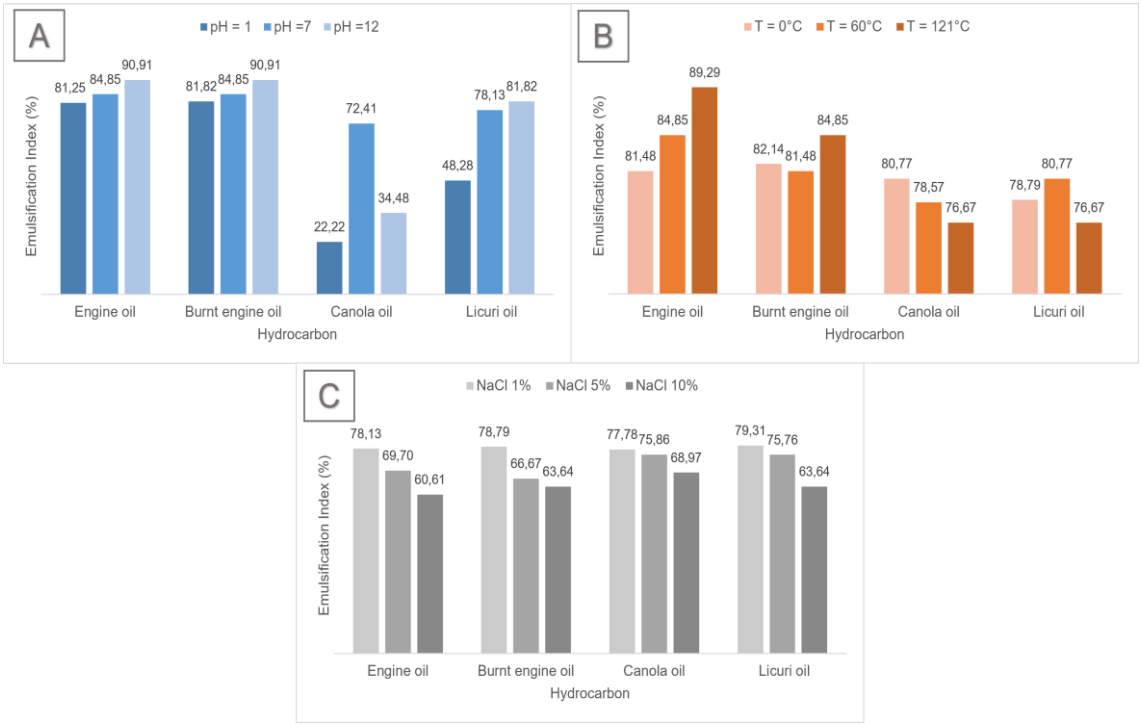

Figure 2 Stability of hydrocarbons emulsification by biosurfactant produced by *C. mogii* UFPEDA 3968 in different conditions of: A) pH; B) Temperature (ºC) and C) NaCl concentration (%).

4 CONCLUSION

This study showcased *C. mogii*'s capacity to produce molecules with surfactant and emulsifying properties when grown in a medium enriched with glucose and Licuri oil. With an emulsification index surpassing 75% under primary test conditions and remaining above 60% under various extreme pH, temperature, and salinity conditions in stability tests, it's evident that the generated biomolecule holds significant promise for diverse applications. Nevertheless, further research is required to optimize the production process and explore its full potential.

REFERENCES

1. SARUBBO, L. A., SILVA, M. DA G. C., DURVAL, I. J. B., BEZERRA, K. G. O., RIBEIRO, B. G., SILVA, I. A., TWIGG, M. S. & BANAT, I. M. 2022. Biosurfactants: Production, properties, applications, trends, and general perspectives. Biochem Eng J 181, 108377. doi:10.1016/j.bej.2021.108377
2. RIBEIRO, B. G., GUERRA, J. M. C. & SARUBBO, L. A. 2020. Biosurfactants: Production and application prospects in the food industry. Biotechnol Prog 36, Preprint at https://doi.org/10.1002/btpr.3030.
3. DA SILVA, R. R., SANTOS, J. C. V., MEIRA, H. M., ALMEIDA, S. M., SARUBBO, L. A. & LUNA, J. M. 2023. Microbial Biosurfactant: Candida bombicola as a Potential Remediator of Environments Contaminated by Heavy Metals. Microorganisms 11, 2772. doi:10.3390/microorganisms11122772.
4. COOPER, D. G. & GOLDENBERG, B. G. 1987. Surface-active agents from two Bacillus species. Appl Environ Microbiol 53.
5. TAVARES, J., ALVES, L., SILVA, T. P. & PAIXÃO, S. M. 2021. Design and validation of an expeditious analytical method to quantify the emulsifying activity during biosurfactants/bioemulsifiers production. Colloids Surf B Biointerfaces 208.
6. MULLIGAN, C. N. 2005. Environmental applications for biosurfactants. Environmental Pollution 133.
7. BEZERRA, K. G. O., DURVALA, I. J. B., SILVA, I. A., ALMEIDA, F. C. G., MELO, Y. T. F., RUFINO, R. D. & SARUBBO, L. A. 2020. Emulsifying capacity of biosurfactants from chenopodium quinoa and pseudomonas aeruginosa UCP 0992 with focus of application in the cosmetic industry. Chem Eng Trans 79.
8. ARAUJO, J. M. M., MONTEIRO, J. M., SILVA, D. H. DOS S., VEIRA, A. K., SILVA, M. R. C., FERRAZ, F. A., BRAGA, F. H. R., SIQUEIRA, E. P. DE & MONTEIRO, A. DE S. 2023. Candida krusei M4CK Produces a Bioemulsifier That Acts on Melaleuca Essential Oil and Aids in Its Antibacterial and Antibiofilm Activity. Antibiotics 12.
9. BHAUMIK, M., DHANARAJAN, G., CHOPRA, J., KUMAR, R. R., HAZRA, C. & SEN, R. 2020. Production, partial purification and characterization of a proteoglycan bioemulsifier from an oleaginous yeast. Bioprocess Biosyst Eng 43.
10. JOSHI-NAVARE, K., KHANVILKAR, P. & PRABHUNE, A. 2013. Jatropha oil derived sophorolipids: Production and characterization as laundry detergent additive. Biochem Res Int. doi:10.1155/2013/169797.

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*.