OPTIMIZING BIOSURFACTANT PRODUCTION FROM NOVEL *Paenibacillus* AND *Psychrobacillus* STRAINS

Isabella C. V. Argentino, Mateus G. de Godoy[1], Lucy Seldin[2] & Diogo de A. Jurelevicius[1]*

[1] *Laboratório de Biotecnologia e Ecologia Microbiana, Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.*
[2] *Laboratório de Genética Microbiana, Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.*
** Corresponding author's email address: diogoj@micro.ufrj.br*

ABSTRACT

The search for sustainable alternatives to synthetic surfactants has intensified interest biosurfactants and bioemulsifiers. Despite their advantages, large-scale production remains costly. Therefore, discovering new bacterial species and optimizing production conditions are crucial. This study investigates the biosurfactant production capabilities of newly isolated bacterial strains LABEM I01, LABEM I02 (*Paenibacillus* spp.), and LABEM I03 (*Psychrobacillus* sp.), optimizing production conditions and characterizing their genomes and metabolism. Emulsification and drop collapse tests confirmed biosurfactant production. Using Central Composite Design (CCD), optimal production conditions were identified: LABEM I01 (34°C, pH 9, 2% salinity), LABEM I02 (34°C, independent of pH and salinity), and LABEM I03 (34°C, pH 5, independent of salinity). HPLC analysis revealed LABEM I01 and LABEM I02 produce fengycin, while LABEM I03 does not produce surfactin or fengycin, highlighting the impact of cultivation conditions. Genome analysis confirmed the strains as novel species with biosurfactant-related genes. This research underscores the importance of optimizing growth conditions to enhance biosurfactant production, supporting their use as sustainable surfactant alternatives.

Keywords: Experimental planning. High Performance Liquid Chromatography (HPLC). Lipopeptídeos. Biotechnology.

1 INTRODUCTION

Biosurfactants and bioemulsifiers are microbial surfactants that lower surface and interfacial tensions, due to their amphipathic properties, enabling better interaction between different phases[1]. Biosurfactants feature hydrophilic and hydrophobic components that allow them to perform various tasks, whereas bioemulsifiers, having a larger molecular mass, stabilize emulsions[2]. Both offer several environmental and performance advantages over synthetic surfactants, including biodegradability, lower toxicity and effectiveness under extreme conditions[3]. The demand for these compounds is increasing due to their advantages and the desire for more sustainable alternatives. Despite this, the bulk of commercial surfactants still come from petroleum, which poses environmental and health risks[4]. Large-scale production of biosurfactants remains expensive, necessitating innovative strategies to optimize microbial production and reduce costs[5]. Ongoing research into diverse microbial strains and production techniques is essential for enhancing the use of biosurfactants and discovering new genes and metabolic pathways involved in their synthesis.

2 MATERIAL & METHODS

Our group has already isolated and identified the bacterial strains used in this investigation. LABEM I01 (*Paenibacillus* sp.) and LABEM I02 (*Paenibacillus* sp.) were isolated from the soil of Grumari beach's sandbank, while LABEM I03 (*Psychrobacillus* sp.) was isolated from the marine sediment of the same beach in Rio de Janeiro, Brazil (23°2'59"S 43°31'35"W). These bacteria were cultured in Trypticase Soy Broth (TSB) medium. Initially, a 10 µl aliquot of each bacterial strain was transferred to Erlenmeyer flasks containing 100 ml of TSB and incubated at 30°C and 170 rpm for 24 hours. Subsequently, 10 ml of each pre-inoculum was transferred to Erlenmeyer flasks containing 110 ml of TSB medium (totaling 120 ml) and incubated under the same conditions for 48 hours. After growth, cultures were centrifuged at 10,000 x g and 4°C for 20 minutes. Surface tension reduction was measured. To assess the generation of lipopeptide class biosurfactants by the bacteria LABEM I01, LABEM I02, and LABEM I03, a lipopeptide extraction step was performed using a modified method of Tiossi et al.[6]. The extracted residue was solubilized in 2 ml of methanol and analyzed using high-performance liquid chromatography (HPLC). For emulsification and surface tension reduction experiments, 20 ml of the supernatant was used. Central Composite Design (CCD) analyses were conducted to determine the optimal conditions for biosurfactant and bioemulsifier production, evaluating temperature (26°C to 34°C), pH (5.0 to 9.0), and salinity (2% to 4%). Statistical analysis of the CCD was performed using Protimiza software (http://experimental-design.protimiza.com.br). In all cases, the production of biosurfactants and/or bioemulsifiers was evaluated using the emulsification test and the pendant drop method, which determined the reduction in water surface tension, using a goniometer.

3 RESULTS & DISCUSSION

Using a 10% of statistical significance (p-value < 0.1), the emulsification test findings did not support an optimal model for bioemulsifier synthesis by LABEM I01, LABEM I02, and LABEM I03. However, ANOVA showed that the model for the surface tension response was predictive, allowing the construction of response surfaces, taking into account the variables (i) pH and salinity, (ii) temperature and pH, and (iii) temperature and salinity (Figures 1A, 2B, and 3C). Based on the findings, it was feasible

to discern a trend indicating increased biosurfactant activity in the supernatant of LABEM I01 bacteria collected at 34 °C, pH 9, and 2% salt.

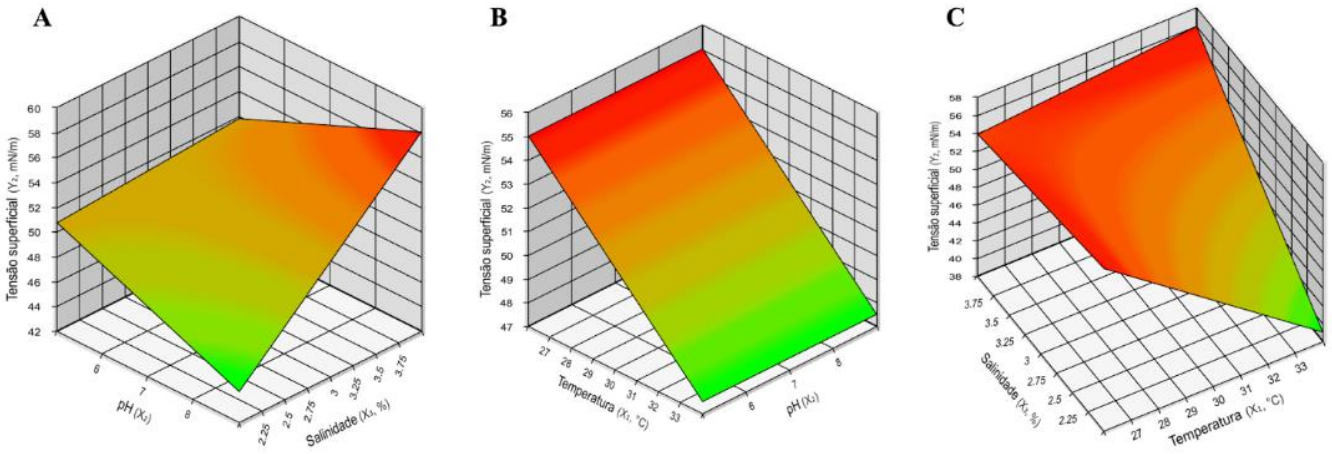

Figure 1 – Response surfaces for reducing the surface tension using the supernatant of LABEM I01 bacteria. (A) Response surface obtained as a function of pH and salinity variables. (B) Response surface obtained as a function of the variables temperature and pH. (C) Response surface obtained as a function of the salinity and temperature variables.

Similarly, a response surface based on the temperature variable was produced based on the response model (surface tension) (Figure 2). A trend suggesting increased biosurfactant activity in the LABEM I02 bacterium supernatant obtained at 34 °C, independent of pH and temperature, was discernible based on the acquired results.

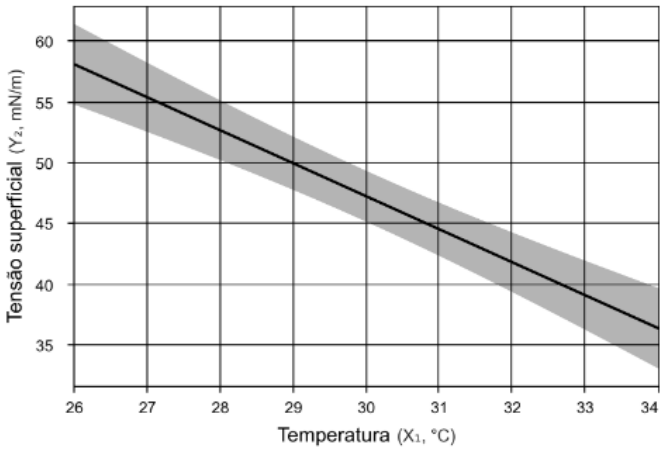

Figure 2 - Reduction in surface tension of the LABEM I02 supernatant obtained as a function of the temperature variable.

For the LABEM I03 strain, a response surface was generated using the model obtained for surface tension, in function of the variables temperature and pH (Figure 3). This method allowed detection of higher biosurfactant activity in the supernatant of the LABEM I03 collected at a temperature of 34 °C and pH 5, without regard to salinity.

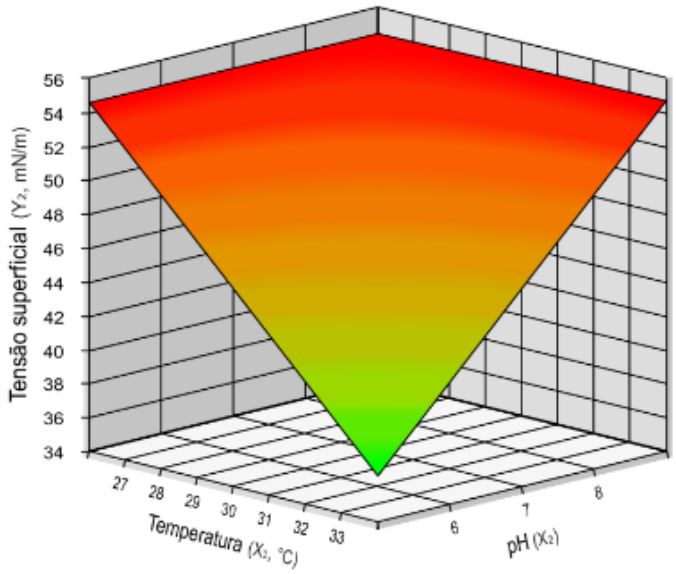

Figure 3 - Response surface for the reduction in surface tension of the LABEM I03 supernatant obtained as a function of the variables temperature and pH.

To determine if the bacteria LABEM I01, LABEM I02, and LABEM I03 produce the biosurfactants surfactin and/or fengycin, high performance liquid chromatography (HPLC) was used. LABEM I01 and LABEM I02 strains displayed chromatographic patterns that were comparable to the chromatographic profile corresponding to fengycin with a retention period between 8 and 9.5 minutes. However, under the evaluated conditions, none of the bacteria produced surfactin.

4 CONCLUSION

This study optimized the growth conditions for LABEM I01, LABEM I02, and LABEM I03 bacteria using Central Composite Design (CCD). The optimal conditions discovered were identified as 34°C, pH 9, and 2% salinity for LABEM I01; 34°C, independent of pH and salinity for LABEM I02; and 34°C and pH 5, independent of salinity for LABEM I03. High Performance Liquid Chromatography (HPLC) analysis revealed that LABEM I01 and I02 produce fengycin, while LABEM I03 does not produce surfactin or fengycin under the tested conditions. These findings underscore the importance of optimizing cultivation conditions to enhance biosurfactant production and promote sustainable alternatives to conventional surfactants. Further research should focus on scaling up production processes and exploring the commercial viability of these biosurfactants.

REFERENCES

1 Sarubbo LA, Silva M da GC, Durval IJB, Bezerra KGO, Ribeiro BG, Silva IA, et al. Biosurfactants: Production, properties, applications, trends, and general perspectives. Biochem Eng J. 2022 Apr;181:108377.

2 Kashif A, Rehman R, Fuwad A, Shahid MK, Dayarathne HNP, Jamal A, et al. Current advances in the classification, production, properties and applications of microbial biosurfactants – A critical review. Adv Colloid Interface Sci. 2022 Aug;306:102718.

3 Santos DKF, Meira HM, Rufino RD, Luna JM, Sarubbo LA. Biosurfactant production from Candida lipolytica in bioreactor and evaluation of its toxicity for application as a bioremediation agent. Process Biochemistry. 2017 Mar;54:20–7.

4 Baccile N, Poirier A, Seyrig C, Le Griel P, Perez J, Hermida-Merino D, et al. Chameleonic amphiphile: The unique multiple self-assembly properties of a natural glycolipid in excess of water. J Colloid Interface Sci. 2023 Jan;630:404–15.

5 Markande AR, Patel D, Varjani S. A review on biosurfactants: properties, applications and current developments. Bioresour Technol. 2021 Jun;330:124963.

6 Tiossi RFJ, Miranda MA, de Sousa JPB, Praça FSG, Bentley MVLB, mcchesney JD, et al. A Validated Reverse Phase HPLC Analytical Method for Quantitation of Glycoalkaloids in Solanum lycocarpum and Its Extracts. J Anal Methods Chem. 2012;2012:1–8.

ACKNOWLEDGEMENTS

To Capes and CNPq for the scholarship and Faperj for funding the project.