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APPLICATION OF GREEN SURFACTANTS TO CONTROL BIOFILM-FORMING MICROORGANISMS

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ABSTRACT

Submerged surfaces in marine environments often face fouling caused by biological communities, leading to significant economic losses. Traditional methods for removing these fouling involve the use of toxic substances in the aquatic ecosystem, which can dead diverse organisms. Surfactants can interact with a diverse array of materials and substances, making them applicable across various fields. Green surfactants (GS), distinct from their petrochemical-derived counterparts, are derived from vegetable or microbial sources. They possess characteristics such as low toxicity, biodegradability, stability under extreme environmental conditions, low critical micelle concentration levels, antimicrobial and antibiofilm properties. This study aimed to assess the effectiveness of GS in controlling microorganisms responsible for biofouling. Marine microorganisms were isolated and cultivated in a marine environment supplemented with seawater. Later, strains capable of forming biofilms were identified using a culture medium containing Congo red and sucrose. Then, two green surfactants, glyceryl laurate and a glycolipid surfactant derived from *Starmerella bombicola* ATCC® 22214™, were tested against these biofilm-forming strains. Both surfactants demonstrated significant antibiofilm activity against all tested strains of biofilm-forming microorganisms. In conclusion, the evaluated surfactants present a viable solution for addressing biofouling issues in marine environments, offering a more environmentally friendly approach compared to conventional methods.

Keywords: Biosurfactant. Glycolipid. Microfouling. Antibiofilm.

1 INTRODUCTION

Biofouling is the colonization of submerged surfaces by living organisms. It undergoes several formation stages, resulting in the development of bacteria and other marine life on the surfaces of different materials, causing detrimental effects on facilities, equipment, and objects used by humans[1]. One of these stages is forming biofilms, an adaptive mechanism and survival tactic used by bacteria. The biofilm produces an extracellular polymeric substance (EPS) that shields the bacteria within, protecting them from harmful environmental influences and immune responses. To prevent biofilm formation on marine surfaces, anti-fouling protections containing biocides are used, which are harmful to the local ecosystem[2,3,4].

There is significant concern about the biocides commonly used in commercial anti-fouling protection systems due to the high concentration found in coastal areas and the potential harm they can cause to marine organisms[3].

Green surfactants, like synthetic surfactants, are biologically sourced chemical compounds that exhibit amphipathic and surfactant properties acting on the surface of different materials[5] and key attributes capable of replacing synthetic versions[6]. Such multifunctionality allows them to interact with various substances, making them suitable for diverse applications. They offer several advantages over synthetic surfactants, including low toxicity, biodegradability, activity, and resistance under extreme conditions[7]. Moreover, they have proven to be effective inhibitors of microbial adhesion and biofilm formation[8,9,10,11].

2 MATERIAL & METHODS

Collection, isolation, cultivation, and maintenance of marine microorganisms

Marine microbial strains were obtained from seawater collected in a sterilized container, as well as by swabbing the surface of encrusting organisms' shells, such as barnacles and mussels, using sterile swabs in a medium-free tube with a plastic shaft. This collection took place at Piedade Beach, in the Metropolitan Region of Recife, Pernambuco, Brazil.

The isolation, stock culture, and maintenance of the strains were carried out using SSPA medium (Sea salt, peptone, and Agar-Sigma-Aldrich). The cultures were maintained at 28°C ± 2 in an incubator for 24 hours.

After the microorganisms grew in the SSPA medium, these colonies were transferred to a medium for detecting biofilm-producing strains. Upon selection, the biofilm-producing strains were transferred to a new SSPA medium, incubated under the same conditions mentioned, and after growth, stored in an inclined tube at -4°C to proceed with the next experimental steps.

Qualitative detection of biofilm-forming strains

Biofilm-producing strains were selected using the qualitative Congo Red Agar (CRA) method. Nutrient agar supplemented with sucrose (50 g/L) and Congo red dye (0.8 g/L) was employed for this method. Congo red was prepared as a concentrated aqueous solution and autoclaved separately from the other constituents. This solution was added to the mixture when cooled to 55°C.

Microbial cells isolated from Piedade Beach were inoculated onto CRA medium and aerobically incubated at 28°C ± 2 for 24-48 hours. Each plate was inoculated with up to two strains of microorganisms isolated from Piedade Beach, Pernambuco state, Brazil.

Green surfactants

The green surfactants used in this study were glyceryl laurate and a glycolipidic surfactant extracted from *Starmella bombicola* ATCC® 22214™, both provided by the Bioengineering Laboratory of UNICAP and the Biotechnology Laboratory of IATI respectively.

Evaluation of Antibiofilm Action of Biosurfactants

The tested strains were the selected ones after the biofilm formation screening. These strains were cultured and diluted to an OD 600 nm of 0.2 with SSP growth medium (Sea Salt Peptone).

Plates were prepared containing SSP medium with sucrose (50 g/L) and Congo red, with the separate addition of glyceryl laurate and glycolipidic surfactant solutions. A total of 66 plates were organized for each type of surfactant (22 for glyceryl laurate, 22 for Sophorolipid, and 22 for SDS). Plates were inoculated with at least two different microbial strains at the indicated dilution. Plates were incubated for 24-48 hours at 37°C. After this period, plates were checked for growth and biofilm formation. Plates untreated with the tested green surfactants served as controls.

3 RESULTS & DISCUSSION

Obtaining and Isolation of Marine Biofilm-Producing Strains

The process of isolating marine microbial strains from encrusted areas, that is, superficially covered with algae and barnacles, allowed the growth of more than 50 colonies. As shown in Figure 1, these colonies can visually be different species. Colonies for the following tests were selected from these marine strains through visual selection based on the color formed in the Congo red culture medium. This culture medium, in addition to the Congo red reagent, contained sugar sucrose and agar. The strains that grew in this medium and that had dark biomass in consistency were considered biofilm producers, while colonies with red biomass were estimated as non-biofilm producers (Figure 2).

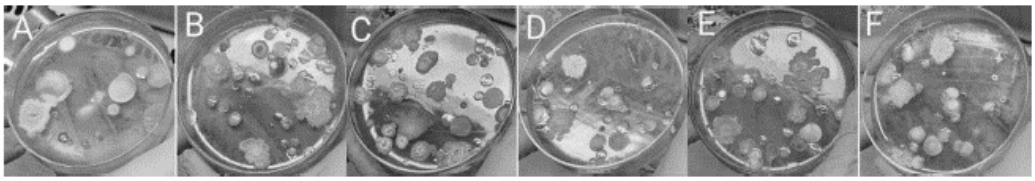

Figure 1 Results of plates with marine medium SSPA after incubation. A and B - microorganisms isolated from seawater; C and D - microorganisms isolated from the surface of algae-encrusted reefs; and E and F - microorganisms isolated from the surface of barnacle-encrusted reefs.

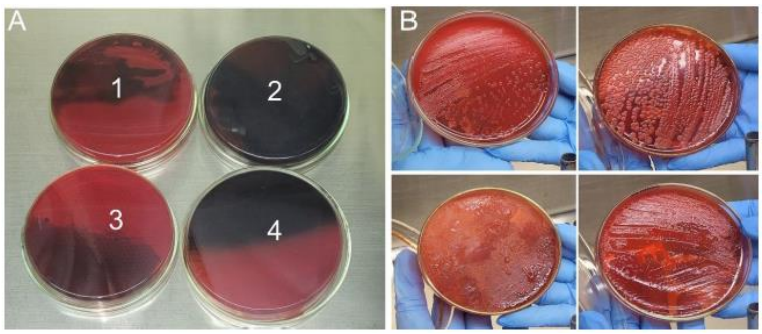

Figure 2 Results of qualitative testing on some of the plates with CRA medium containing sucrose after incubation for the detection of marine biofilm-forming strains. (A) 1 – 4 plates where at least one biofilm-forming strain was observed, (B) plates where no biofilm formation was observed by any strain.

In Figure 2A, on the plates identified by 1, 3 and 4, biofilm formation occurred in at least one of the strains that were obtained from the process of isolating marine microbial strains. Still in Figure 2A, the plate indicated by number 2, the two inoculated strains formed biofilms, evidenced by the dark color across the entire surface of the medium. On the contrary, in the plates in Figure 2B, all isolated strains did not show dark color, in this case, it can be inferred that there was no formation of biofilms by these strains. In this way, through this simple test it was possible to identify 22 biofilm-producing strains.

Evaluation of the anti-biofilm action of natural surfactants

In Figure 4, the results of the behavior of the strains that were selected as biofilm formers when in the presence of the surfactants evaluated here are presented. In most plates, biofilm formation by the strains was inhibited by the presence of glycolipid and laurate surfactants in the culture medium. On plates with yellow arrows, weak or absent microbial growth is indicated. Indicating that, in addition to interfering with biofilm production, the growth of the microorganism was also inhibited. However, on plate number 4, one of the strains showed weak growth, but produced biofilm. In this isolated case, it can be inferred that the biosurfactant, at the concentration tested in the present assay, did not inhibit the growth of this microorganism and it was still capable of producing the biofilm as a protective barrier.

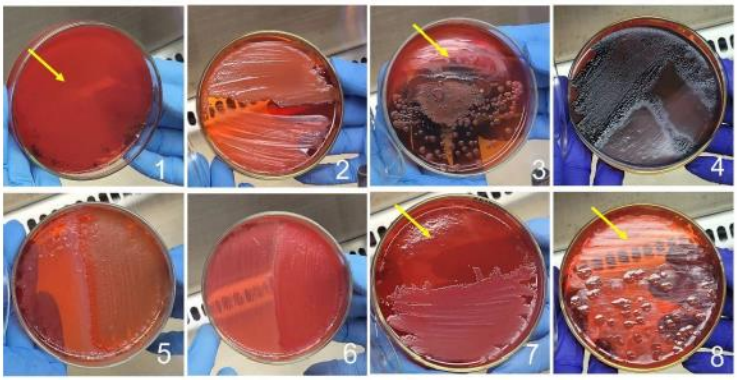

Figure 4 Results of plates with CRA medium containing sucrose and the surfactants glycolipidic and glyceryl laurate after 48 hours of incubation.

Of the 22 strains selected for biofilm formation, approximately 77.27% lost the ability to form them only in the presence of surfactants (sophorolipid and glyceryl laurate). Meanwhile, 100% were inhibited by SDS. The biobased surfactants glyceryl laurate and the synthetic surfactant SDS affected the production of bacteria's EPS (exopolysaccharides).[12] The glycolipid surfactant produced by *Serratia marcescens* was able to disrupt pre-formed biofilms of the pathogens *Candida albicans* and *Pseudomonas aeruginosa* and the marine biofouling bacterium *Bacillus pumilus*. Indicating that the glycolipid derived from *S. marcescens* could thus serve as a potential anti-biofilm agent.[13] Although the type of glycolipid surfactant was not identified by the producing strain in this study, *Starmerella bombicola* usually produces sophorolipids in the culture medium used. Thus, the antibiofilm action of sophorolipid-type glycolipids may have occurred by altering the structure of the bacterial cell membrane, resulting in increased permeability and disruption of membrane integrity.

4 CONCLUSION

The culture medium with Congo red dye is an inexpensive medium that could contribute to the identification of biofilm-producing strains. The strains identified as biofilm producers had their growth and consequently the production of their biofilm inhibited when in contact with the green surfactants evaluated in this research. This simple test shows the importance of green surfactants as bioproducts that can contribute to the control of biofilms in different environments, such as submerged structures in the marine environment to the hospital environment.

REFERENCES

[1] VALCHEV, I., CARADDU, A., KALIKATZARAKIS, M., GEERTSMA, R., ONETO, L. Ocean Eng., v. 251, n. January, p. 110883, 2022.
[2] ALLEGRONE, G., CERESA, C., RINALDI, M., FRACCHIA, L. Pharmaceutics., v. 13, p. 1172, 2021.
[3] AMARA, I., MILED, W., SLAMA, R. B., LADHARI, N. Environ. Toxicol. Farmacol., v. 57, n. December 2017, p. 115–130, 2018.
[4] KYEI, S. K.; DARKO, G.; AKARANTA, O. J. Coat. Technol. Res., v. 17, n. 2, p. 315–332, 21 mar. 2020.
[5] CZUBA, U., QUINTANA, R., LASSAUX, P., BOMBERA, R., CECCONE, G., BAÑULS-CISCAR, J., MORENO-COURANJOU, M., DETREMBLEUR, C., CHOQUET, P. Colloids Surf. B: Biointerfaces, v. 178, p. 120–128, 1 jun. 2019.
[6] ZHU, Z., WEN, Y., YI, J., CAO, Y., LIUC, F., MCCLEMENTS, D. J. J. Colloid Interface Sci., v. 536, p. 80–87, 2019.
[7] SARUBBO, L. A., SILVA, M. G. C., DURVAL, I. J. B., BEZERRA, K. G. O., RIBEIRO, B. G., SILVA, I. A., TWIGG, M. S., BANAT, I. M. Biochem. Eng. J., v. 181, p. 108377, abr. 2022.
[8] ABDALLAH, D. B., TOUNSI, S., GHARSALLAH, H., HAMMAMI, A., FRIKHA-GARGOURI, O. Environ. Sci. Pollut. Res. Int., v. 25, n. 36, p. 36518–36529, 2018.
[9] ABDELLI, F. JARDAK, M., ELLOUMI, J., STIEN, D., CHERIF, S., MNIF, S., AIFA, S. Biodegradation, v. 30, n. 4, p. 287–300, 2019.
[10] KHALID, H. F., TEHSEEN, B., SARWAR, Y., HUSSAIN, S. Z., KHAN, W. S., RAZA, Z. A., BAJWA, S. Z., KANARAS, A. G., HUSSAIN, I., REHMAN, A. J. Hazard. Mater., v. 364, p. 441–448, 2019.
[11] OHADI, M., FOROOTANFAR, H., DEHGHANNOUDEH, G., ESLAMINEJAD, T., AMERI, A., SHAKIBAIE, M., ADELI-SARDOU, M. Microb. Pathog., v. 138, p. 103806, jan. 2020.
[12] SILVA, M. DA G. C., SILVA, M. E. P., MEDEIROS, A. O., MEIRA, H. M., SARUBBO, L. A. Surfaces, v.5, p. 383–394, 2022.
[13] DUSANE, D. H., PAWAR, V. S., NANCHARAIAH, Y. V., VENUGOPALAN, V. P., KUMAR, A. R., ZINJARDE, S. S. Biofouling, v. 27, p. 645–654, 2011.

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