BIOTECHNOLOGICAL POTENTIAL OF BACTERIA ISOLATED FROM MARINE SANDY SEDIMENT CONTAMINATED IN THE PRODUCTION OF BIOSURFACTANTS

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ABSTRACT

Oil pollution is a critical environmental concern, necessitating the search for increasingly effective technological solutions to mitigate its catastrophic impacts. This scenario has driven the demand for green technologies, promoting the search for new remediation strategies, such as the use of biosurfactants. Biosurfactants are compounds synthesized by microorganisms that possess properties similar to chemical surfactants, such as the ability to reduce surface tension and form emulsions. In this context, the present study aimed to isolate bacteria with potential for producing biosurfactants and bioemulsifiers from a highly impacted area in Guanabara Bay, Rio de Janeiro. Nine different bacterial strains were isolated, all characterized as aerobic, Gram-positive, spore-forming bacteria. The bacteria were cultured in Luria-Bertani Broth LBB (pH 7.0) and Nutrient Broth NB (pH 7.0) under agitation at 160 rpm and 30°C / 48h. After this period, the whole content was centrifuged, and the potential for biosurfactant and bioemulsifier production was evaluated. The BGF1-1LBB and BGF1-2NB strains were capable of producing biosurfactants, while the BGF3-1aNB and BGF1-2NB strains produced bioemulsifiers, presenting an emulsification index of 92.4% and 85.4% after 24h. The bacterial strains isolated from the contaminated area in Guanabara Bay demonstrated biotechnological potential for use in bioremediation processes of environments contaminated with oil and its derivatives.

Keywords: Oil Biodegradation, Bacteria, Biosurfactant Production, Used Ship Oil.

1 INTRODUCTION

Environmental contamination caused by petroleum-derived products such as diesel, gasoline, lubricating oils, and crude oil has gained significant ecological attention in recent years[1]. This pollution is caused by various activities, including maritime transport, with its over 8,000 oil tankers and thousands of other medium and large vessels, which require tank cleaning in port areas, resulting in the intentional pollution of approximately 5 million tons of hydrocarbons per year[2,3,4]. Other polluting activities involve the discharge of untreated effluents, offshore and onshore oil industry operations, and accidental spills. These leaks and spills are expected during the exploration, production, refining, transportation, processing, as well as storage of petroleum and petroleum products[1,5]. Among the petroleum-derived pollutants released into the marine environment, we highlight lubricating oil from ships and boats, which may contain alkanes, alkenes, and aromatic groups, as well as various hepatotoxic additives that cause severe health damage, including the possibility of death[2,3,4,6]. These pollutants drastically harm marine ecosystems and have profound socioeconomic impacts, affecting sectors such as maritime sports, tourism, and fishing, which depend on clean waters[3,4].

The continuous development and improvement of technologies that promote the sustainability of natural resources are widely supported and encouraged by various environmental protection entities and conscious governments. Several environmental technologies, such as the use of bioremediation tools, less toxic dispersants, and the development of containment barriers, are examples of innovations that can significantly contribute to the protection of marine and coastal ecosystems[1,2,7]. Among the bioremediation strategies used to degrade petroleum components, biosurfactants stand out. Biosurfactants are compounds synthesized by microorganisms with surfactant properties, offering several advantages compared to chemical surfactants, such as high surface tension reduction, thermal stability, low toxicity, biodegradability, and stability under extreme pH values[8,9]. The applicability of biosurfactants is diverse, primarily due to their low toxicity and biodegradability, making them suitable for use in the food, pharmaceutical, cosmetic, and agricultural industries. Many bacterial species from various environments have been isolated and used as biodegraders of petroleum hydrocarbons, capable of producing different biomolecules such as enzymes, biosurfactants, and bioemulsifiers that aid in the uptake and biodegradation of crude oil and petroleum hydrocarbons, which can be used in bioremediation strategies to recover environments polluted by petroleum and its derivatives[10]. However, no single species can completely degrade complex petroleum-derived hydrocarbons. About 175 bacterial genera are reported in the literature as capable of utilizing hydrocarbons, among which species such as *Achromobacter* sp., *Actinobacteria* sp., *Arthrobacter aurescens*, *Bacillus* sp., *Coprothermobacter* sp., *Desulfitobacter* sp., *Desulfosporosinus* sp., *Gammaproteobacteria* sp., *Mycobacterium givum*, *Ochrobactrum* sp., *Pantoea agglomerans*, *Pseudomonas* sp., *Rhodococcus erythropolis*, and *Stenotrophomonas acidaminiphila*[1,9,10] are highlighted. Therefore, this study aimed to evaluate the production of biosurfactants and bioemulsifiers from bacteria isolated from a contaminated marine environment and their potential for biodegradation of ship lubricating oil.

2 MATERIAL & METHODS

2.1 Collection of Environmental Samples and Isolation of Microorganisms

A collection was carried out at multiple points on Fundão Island (22°52'09.0"S 43°12'51.5"W) in Guanabara Bay (Rio de Janeiro), in front of the Porto Brasco, an area contaminated by petroleum-derived sources. Using a shovel, sediment samples were taken from the contaminated area, removing the top 10 cm layer to avoid contamination by non-native microorganisms. The samples were stored in a properly sterilized container and transported to the laboratory in a thermal bag for technical isolation procedures. Microorganism isolation was conducted using the serial dilution technique, where 10 g of sediment was transferred to 250 mL Erlenmeyer flasks containing 90 mL of sterile 0.85% (w/v) saline solution. After 20 minutes of shaking at 150 rpm at room temperature, a 1.0 mL aliquot was transferred to a tube containing 9.0 mL of sterile 0.85% saline solution, homogenized in a vortex for 30 seconds, and a new 1.0 mL aliquot from tube 1 was transferred to tube 2. This procedure was repeated up to tube 4. After this, 0.1 mL aliquots were transferred to Petri dishes containing nutrient agar (beef extract 1.0 g/L, yeast extract 2.0 g/L, peptone 5.0 g/L, NaCl 5.0 g/L, bacteriological agar 18.0 g/L, pH 7.0) using the spread plate technique, in triplicate. The plates were incubated at 32°C for 48 hours, after which microbial colony counts were performed. The colonies were then transferred to another plate containing TSA medium (tryptone 15.0 g/L, papaic soy digest 5.0 g/L, sodium chloride 5.0 g/L, pH 7.0) using the streak plate technique to obtain pure cultures, and incubated for 48 hours at 32°C. After confirming the purity of the microbial strains, they were preserved in 30% glycerol at -20°C.

2.2 Morphological Identification of Microbial Strains

The microbial strains were inoculated on Petri dishes with nutrient agar for 24 hours at 32°C to observe the macroscopic characteristics of the colonies formed. Subsequently, the microscopic characteristics were observed using the Gram staining technique.

2.3 Submerged Fermentation

Bacterial strains were initially grown on nutrient agar for 48 hours at 32°C and then diluted in sterile 0.85% saline solution until the cell concentration was standardized using the MacFarland scale (No. 3). The bacteria were then inoculated into two fermentation systems: (i) Luria-Bertani broth LBB (pH 6.5) and (ii) Nutrient broth NB (pH 6.5) in duplicate systems. The fermentation systems were incubated at 32°C for 48 hours with orbital shaking at 160 rpm. After this period, the entire content was collected, centrifuged at 1,960 g for 10 minutes, and stored in flasks at 4°C for later analysis.

2.4 Oil Displacement Test

For the oil displacement test, 100 µL of the different supernatants obtained from the two submerged fermentation systems (i and ii) were individually added to 1.0 mL of ship lubricating oil (slowly added to form an oil film on water) in 20.0 mL of distilled water placed in a Petri dish base (10 x 2 cm). The result was considered positive when a halo was observed within the oil drop, indicating oil dispersion. The result was considered negative when the supernatant drop only dissolved in the water without altering the oil film. Distilled water was used as a negative control, and a 5% (v/v) neutral detergent solution was used as a positive control[11].

2.5. Emulsification Test

The emulsification capacity of bacterial strains isolated from Guanabara Bay (Rio de Janeiro) was evaluated to verify the production of biosurfactants. For this, 1.0 mL of each supernatant obtained from the two submerged fermentation systems (i and ii) was individually added to a 15 mL Falcon tube containing 1.0 mL of ship lubricating oil and 1.0 mL of distilled water. The tubes were vortexed for 2 minutes, and the heights of the total column and the foam were measured in mm. The system was left undisturbed for 48 hours to check the stability of the formed foam. The emulsification potential was verified by the emulsification index after 24 h and 48 h, determined by the following formula (1):

$$E = \frac{\left(height\ of\ the\ emulsified\ layer\ (mm)\right)\ x\ 100}{total\ height\ (mm)} \quad (1)$$

3 RESULTS & DISCUSSION

3.1 Collection of Environmental Samples and Isolation of Microorganisms

Guanabara Bay is a region located in the metropolitan area of Rio de Janeiro, surrounded by seven municipalities and various islands, with the largest being Paquetá Island, Governador Island, and Fundão Island, a complex of reclaimed islands. It is a densely urbanized and industrialized area, presenting a highly eutrophic environment due to high loads of untreated pollutants being discharged. In this scenario, the laboratory team collected contaminated marine sediment in the region of Fundão Island (22°52'09.0"S 43°12'51.5"W) in front of the Brasco port (Figure 1). After the sediment was collected at a depth of 10-20 cm and processed in the laboratory using the serial dilution technique, around nine colonies with differentiated morphological aspects were detected and isolated after 48 hours of incubation at 30°C on nutrient agar medium (pH 7.0).

The isolated bacteria were identified morphologically by observing both macroscopic aspects (colony characteristics) and microscopic aspects (cellular characteristics through Gram staining). All nine strains were identified as Gram-positive rod-shaped bacteria (short and long) forming elliptical endospores, suggesting they belong to the *Bacillus* genus.

3.2 Oil Displacement and Emulsificant Test

The ability to promote the dispersion of the oil film on the water was observed in seven of the nine isolated bacteria (Figure 2). Two different culture media were evaluated for biosurfactant production: LBB medium and NB medium. Of the seven positive strains, only the strains BGF1-1LBB, BGF1-2NB and BGF2-1LBB were able to promote a reduction in the oil/water surface tension, causing an oil drop displacement greater than 3.0 cm in diameter (Figure 2). Various reports in the literature indicate

that different species of *Bacillus* and *Pseudomonas* are capable of producing surface displacement of oil on water through the production of biosurfactants[1,2,9].

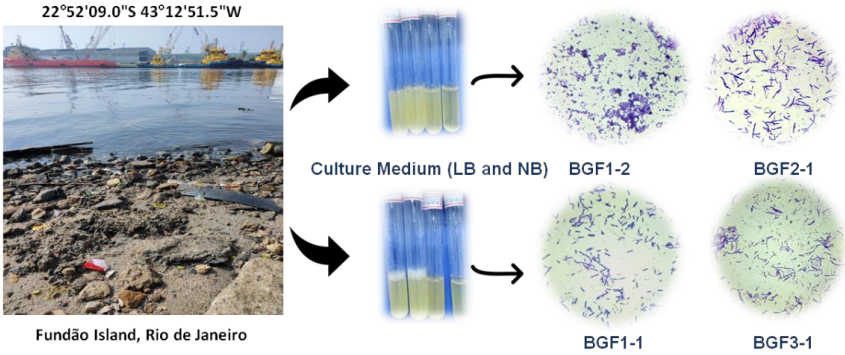

Figure 1 Isolation and Morphological Identification of Bacterial Strains from Contaminated Sediment of Guanabara Bay, Rio de Janeiro

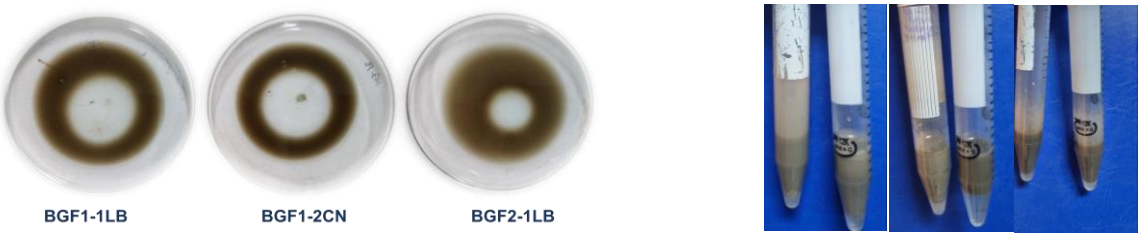

Figure 2 Evaluation of the results on oil displacement and the oil emulsification test using the supernatants from the growth media (LBB and NB) of the bacterial strains isolated from Guanabara Bay, RJ

All nine isolated bacteria showed positive results for emulsification. However, when the emulsification index was evaluated after 24 and 48 hours in the two culture media used (LBB and NB), only three strains had an EI24 above 50% after 24 hours (Table 1). The foaming action is an important characteristic of biosurfactants, aiding in the biodegradation of oils by reducing interfacial and surface tensions[2,9]. In the petroleum industry, biosurfactants can be applied in enhanced oil recovery (MEOR), oil spills, cleaning of oil-contaminated ships, viscosity control, oil emulsification, and crude oil removal from sludge[9].

Table 1 Emulsification Index (values express in *mm*) from bacterial strains isolated from marine sediment contaminated, after 24 and 48 hours.

Bacterial Strain	Medium	T_{24}	T_{48}	Total	EI_{24}	EI_{48}
BGF1-3A	NB	31.0 ± 2.0	31.0 ± 2.0	32.0 ± 2.0	**92.4%**	**92.4%**
BGF1-3A	LBB	15.5 ± 0.5	15.5 ± 0.5	30.5 ± 0.5	50.8%	50.8%
BGF1-2	NB	26.5 ± 1.5	27.0 ± 2.0	31.0 ± 1.0	**85.4%**	**87.1%**
BGF1-2	LBB	20.0 ± 6.0	20.0 ± 6.0	34.0 ± 0.5	58.8%	58.8%
BGF1-1	NB	19.5 ± 2.0	15.5 ± 2.0	32.0 ± 1.0	60.9%	48.4%
BGF1-1	LBB	19.0 ± 2.0	18.0 ± 2.0	31.5 ± 1.5	60.3%	57.1%

4 CONCLUSION

Guanabara Bay is an environment of great biodiversity, despite being impacted by anthropogenic pollution. Various studies indicate the presence of different microorganisms with the potential to degrade petroleum derivatives, such as the present study which isolated nine bacterial strains that showed potential to produce biomolecules with biosurfactant and bioemulsifying action. All strains were Gram-positive, aerobic, endospore-forming rods.

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