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Production of biosurfactants by bacteria collected in soil contaminated by diesel oil on the Antarctic Continent

Produção de biossurfactantes por bactérias coletadas em solo contaminado por óleo diesel no Continente Antártico

Producción de biosurfactantes por bacterias recolectadas en suelos contaminados por petróleo diesel en el Continente Antártico

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Abstract

Currently, the whole world strives for a sustainable development to avoid the depletion of natural resources. Therefore, the search for new biotechnological products such as biosurfactants has generated interest due to its low toxicity, biodegradability and ecological acceptability. Furthermore, biosurfactants have several applications such as emulsification, wettability, foaming, cleaning, and reduction of crude oil viscosity. The objective of this work was to identify the bacteria collected in Antarctica and evaluate their capacity to produce biosurfactants. In this study, bacteria collected in diesel contaminated soil around the Comandante Ferraz Antarctic Station were identified (*Rhodococcus erythropolis*, *Rhizobium radiobacter*, *Microbacterium liquefacien*, *Pseudomonas libanensis* and *Pseudomonas veronii*) and analyses were carried out to confirm whether these bacteria produce biosurfactants. Interestingly, the compounds isolated from the bacteria showed droplet propagation and reduced the surface tension of the water, which are properties of biosurfactants. Furthermore, these compounds showed carbohydrates in their molecular composition. Given these findings, these species have potential application in biotechnological areas, such as bioremediation of environments contaminated by diesel oil and in cosmetic and personal care as emulsifier. In addition, the use of diesel oil waste by bacteria as a carbon source becomes an attractive alternative because it reduces environmental contamination and has a low cost.

Keywords: Bacteria; Antarctica; Biosurfactant; Oil.

Resumo

Atualmente, o mundo inteiro se esforça pelo desenvolvimento sustentável para evitar o esgotamento dos recursos naturais. Portanto, a busca por novos produtos biotecnológicos, como os biossurfactantes, têm gerado interesse devido à sua baixa toxicidade, biodegradabilidade e aceitabilidade ecológica. Além disso, essas moléculas possuem diversas aplicações como emulsificação, molhabilidade, formação de espuma, limpeza e redução da viscosidade do óleo cru. O objetivo deste trabalho foi identificar as bactérias coletas na Antártida e avaliar sua capacidade de produção de biosurfactantes. Neste estudo, bactérias coletadas em solo contaminado por óleo diesel ao redor da Estação Antártica Comandante Ferraz foram identificadas (*Rhodococcus erythropolis*, *Rhizobium radiobacter*, *Microbacterium liquefacien*, *Pseudomonas libanensis* e *Pseudomonas veronii*) e os testes para verificarem se estas bactérias produzem biossurfactante foram feitos. Interessantemente, os compostos isolados das bactérias apresentaram propriedades que permitiram identificá-los como biossurfactantes, pois promoveram a propagação da gota e reduziram a tensão superficial da água. Além disso, estes compostos apresentam carboidratos em sua composição molecular. Diante desses achados, essas espécies apresentam potencial de aplicação em áreas biotecnológicas, como biorremediação de ambientes contaminados por óleo diesel e como emulsificantes em produtos cosméticos e de cuidado pessoal. Além disso, a utilização de resíduos de óleo diesel pelas bactérias como fonte de carbono torna-se uma alternativa atraente para reduzir a contaminação ambiental e apresentar baixo custo.

Palavras-chave: Bactéria; Antártica; Biossurfactante; Óleo.

Resumen

Actualmente, el mundo entero lucha por un desarrollo sostenible para evitar el agotamiento de los recursos naturales. Por ello, la búsqueda de nuevos productos biotecnológicos como los biosurfactantes ha generado interés debido a su baja toxicidad, biodegradabilidad y aceptabilidad ecológica. Además, los biosurfactantes tienen varias aplicaciones, como la emulsificación, la humectabilidad, la formación de espuma, la limpieza y la reducción de la viscosidad del petróleo crudo. El objetivo de este trabajo fue identificar las bacterias recolectadas en la Antártida y evaluar su capacidad para producir biosurfactantes. En este estudio, se identificaron bacterias recolectadas en suelo contaminado con diesel alrededor de la Estación Antártica Comandante Ferraz (*Rhodococcus erythropolis*, *Rhizobium radiobacter*, *Microbacterium liquefacien*, *Pseudomonas libanensis* y *Pseudomonas veronii*) y se realizaron análisis para confirmar si estas bacterias producen biosurfactantes. Curiosamente, los compuestos aislados de la bacteria mostraron propagación de gotas y redujeron la tensión superficial del agua, que son propiedades de los biosurfactantes. Además, estos compuestos presentaban carbohidratos en su composición molecular. Dados estos hallazgos, estas especies tienen aplicación potencial en áreas biotecnológicas, como la biorremediación de ambientes contaminados por gasóleo y en cosmética y cuidado personal como emulsionante. Además, el uso de residuos de gasóleo por bacterias como fuente de carbono se convierte en una alternativa atractiva porque reduce la contaminación ambiental y tiene un bajo costo.

Palabras clave: Bacteria; Antártida; Biosurfactante; Aceite.

1. Introduction

Currently, the whole world strives for a sustainable development to avoid the depletion of natural resources. Therefore, the search for new biotechnological product, such as biosurfactants (BS), has generated considerable interest due to its low toxicity, biodegradability and ecological acceptability (Joshi et al., 2015; Anjun et al., 2016; Jimoh and Lin 2020; Sharma and Sharma, 2020). Biosurfactants can be classified as glycolipids, lipopeptides, phospholipids, fatty acids and polymeric compounds (Dusane et al., 2011; Martins and Martins, 2018). Furthermore, the biosurfactants are capable of reducing the surface tension between liquids (Suthar & Nerurkar, 2016; Bodratti et al., 2017).

Biosurfactants can be produced by microorganisms using various substrates such as hydrophobic mixtures, hydrocarbons, solvents, and industrial residues (Bezza & Chirwa, 2017; Nitschke & Silva, 2018; Phulpoto et al., 2020). Biosurfactants can replace synthetic surfactants in a wide variety of industrial applications, such as detergents, foams, emulsifiers, solubilizers and wetting agents due to their characteristics such as low CMC values, ability to reduce surface/interfacial tension and emulsification properties (Barros et al., 2008; Nitschke & Silva, 2018; Hajimohammadi et al., 2018; Befkadu & Chen, 2018). In addition, biosurfactants can perform several biological properties such as fungicides, insecticides, antivirals, antibacterial, anti-biofilm adhesion, anticancer, and enzyme inhibition (Nithya et al., 2010; Harshada, 2014; Helmy et al., 2011; Varvaresou & Iakovou, 2015; Mnif & Ghribi, 2015; Clements et al., 2019; Pires et al., 2020). Currently, researches have been developed to employ the biosurfactants in ecological scenarios such as the bioremediation of hydrophobic compounds (Yong et al., 2015; Luna et al., 2016; Patel et al., 2019; Jimoh & Lin, 2020; Cazals et al., 2020; Al-Dhabi et al., 2020; Thomas et al., 2021).

Primarily, studies with biosurfactant producing microorganisms have been conducted in terrestrial sources (Das et al., 2010). However, microorganisms outside from terrestrial sources can exhibit extraordinary metabolic and physiological characteristics (Satpute et al., 2010a). Unfortunately, microorganisms outside from terrestrial sources are underexplored and there are few published reports with these microorganisms (Das et al., 2010; Satpute et al., 2010a). Therefore, research is highly relevant to elucidate the biotechnological potential of these microorganisms.

Few studies with microorganisms from the Antarctic continent have been carried out due to the difficulty of accessing this ecosystem. It is noteworthy that oil reservoirs would provide a hydrocarbon-rich environment for the enrichment of several biosurfactant producers (Christofi & Ivshina, 2002). In addition, oil reservoirs in extreme conditions such as high or low temperature could formulate a microbial community that differs from others (Grabowski et al., 2005).

The purpose of this work was evaluated whether bacterial collected in the Antarctic in soil contaminated with diesel oil can produce biosurfactant.

2. Methodology

2.1 Material

Bacteria isolates were collected in contaminated soil with diesel oil around the Comandante Ferraz Antarctic Station (EACF) and kindly provided by Dr. Juliano de Carvalho Cury (Federal University of São João del-Rei).

2.2 Bacteria identification

Bacteria isolated were identified by MALDI-TOFF mass spectrophotometry. This spectrophotometry was performed on the Biotyper Microflex Bruker MALDI-TOFF (Billerica/MA, USA) and the *Escherichia coli* was used to calibrate the equipment. Sample analysis was performed by overlaying its chromatograms with standard chromatograms, taking into account the intensity and number of peaks.

2.3 Production and partial purification of biosurfactants

The production and partial purification of biosurfactants were performed by cultivation of bacterial isolates as described below. Firstly, the bacterial isolates were reactivated in nutrient agar at 15°C for 96 h. During the pre-inoculum, bacterial isolates were cultivated in 125 mL of nutrient broth at 15°C under constant agitation at 180 rpm for 24 h and 68 h. During the inoculum, bacterial isolates were cultured in 125 mL of MSM medium (87.5 g/mL of K2HPO4; 25 g/mL of KH2PO4; 12.5 g/mL of (NH4)2SO4; 12.5 g/ mL of Sodium Citrate; 2.5 g/mL of MgSO4). Only 1% diesel oil was added as a carbon source. After cultivation (and production of biosurfactants), cells were removed by centrifugation (Centrifugal Model

MDW-350 – Biosysten) at 3000 rpm for 30 min, obtaining the clarified extract. Following the production of biosurfactants, they were precipitated by adjusting the pH of the centrifuged and cell-free medium to 2.0, employing a 6.0 M HCl solution. The medium remained incubated overnight in the refrigerator at 4 °C, thus completing the process of partial purification of biosurfactants produced.

2.4 Drop collapse test

The drop collapse test was performed in wells of microplates containing 96 shallow wells. The wells of the caps were greased with 2 μL of 10W-40 motor oil and left to stand for 24 h. Then 5 μL of the sample containing the pre-purified biosurfactant solution was dispensed into each well in triplicate and the droplet spreading was evaluated one minute later. The positive result came when the droplet diameter was larger than that of a deionized water droplet used as a control negative. Tween 80 was used as a positive control (Pires et al., 2020).

2.5 Surface tension assessment

The surface tension of the cell-free samples containing the pre-purified biosurfactant solution was made by the Du Nouy ring method (De Nevers & Grahn, 1991). To this analysis was used a K12 Tensiometer model (Krüss, Hamburg, Germany), in which the platinum ring was immersed in 20 mL of a solution containing the biosurfactants in a room temperature (25 °C). Prior to measuring the surface tension of samples, the tensiometer was calibrated using deionized water whose surface tension value was around 73 mN/m. The culture medium was used as a negative control. The surface tension value was determined in mN/m.

2.6 Emulsification index (E24)

Diesel oil (2 mL) and gasoline (2 mL) was added to 2 mL cell-free samples. After addition, the solution was mixed carefully for 2 min on a vortex and left to stand for 24 h. E24 index was calculated by the percentage of the emulsified layer height (LH) (cm) compared to the total height (TH) of the liquid column (cm) (Cooper & Goldenberg, 1987). Deionized water and Tween 80 were used as negative and positive controls, respectively.

2.7 Biochemical composition of biosurfactant

The carbohydrate content on the chemical composition of purified biosurfactant was determined by the phenol–sulfuric acid method as described previously (Dubois et al., 1956). The D-glucose was used as a standard. Absorbance measurement at 490 nm was performed on the plate reader (Epoch, Biotek).

2.8 Statistical Analyses

Data were analyzed by the ANOVA test followed by the Tukey test. Results were expressed as mean ± standard deviation of the mean. $p < 0.05$ was the level of significance adopted for the analysis. The statistical analyses as well as plotting the graphs were performed on the Graph Pad Prism 5 software.

3. Results

Initially samples of bacteria collected in soil contaminated by diesel oil on the Antarctic Continent were submitted to MALD-TOF mass spectrometry to be identified. The quantity of 9 species and 6 genera of bacteria were identified. However we selected 5 species from 4 genera (*Rhodococcus erythropolis*, *Rhizobium radiobacter*, *Microbacterium liquefacien*, *Pseudomonas libanensis* and *Pseudomonas veronii*) for this study because there was little study with these bacteria in the area of biosurfactants. To avoid repeating the name of bacteria throughout the text we use the code form as can be seen in table 1. After identification, the selected bacterial were cultivated and their fermentation products were collected to carry out the characterization tests.

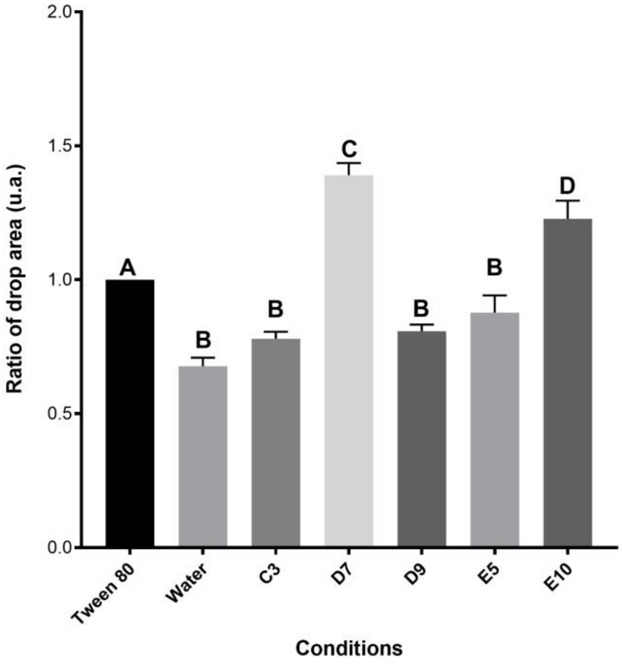
Table 1. Bacteria selected in this study and their respective code.

Bacteria	Code
Rhodococcus erythropolis	C3
Rhizobium radiobacter	D7
Microbacterium liquefaciens	D9
Pseudomonas libanensis	E5
Pseudomonas veronii	E10

Source: Elaborated by the authors.

Drop collapse test of the selected species is shown in Figure 1. Species D7 and E10 showed droplet collapse with a sample test/positive control ratio of 1.4 and 1.3, respectively. Water was used as negative control due to it being a completely oil-immiscible compound and Tween 80 was used as positive control because it is a highly oil-miscible synthetic surfactant.

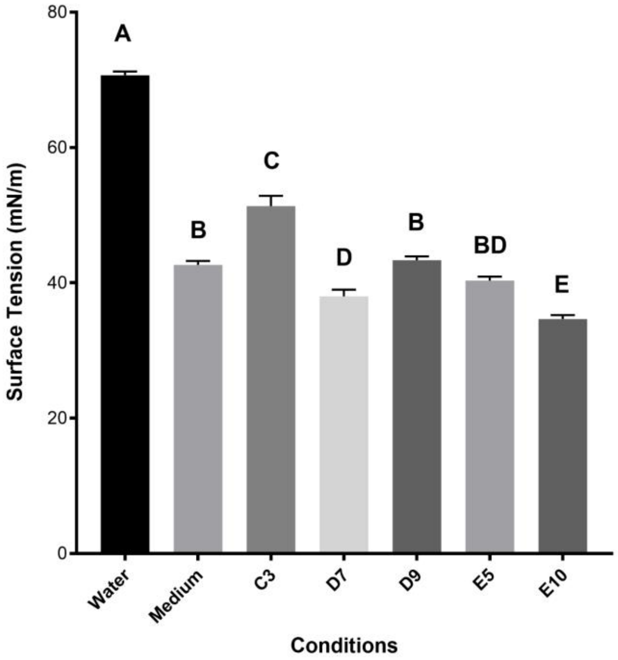
Figure 1. Drop collapse test of isolated compounds of bacterial strains from the Antarctic.



Water was used as negative control and Tween 80 was used as a positive control. Drop collapse analysis was given as a ratio of the areas of the drops formed to the positive control expressed as arbitrary units (u.a.). Results were expressed as mean ± standard deviation of the mean. (n=3). Different letters denote statistically significant differences ($p<0,05$). Source: Elaborated by the authors.

In the Figure 2 is shown the surface tension. Interestingly, Species D7 and E10 produced tenso active compounds capable of reducing the surface tension to 38 mN/m and 34 mN/m, respectively.

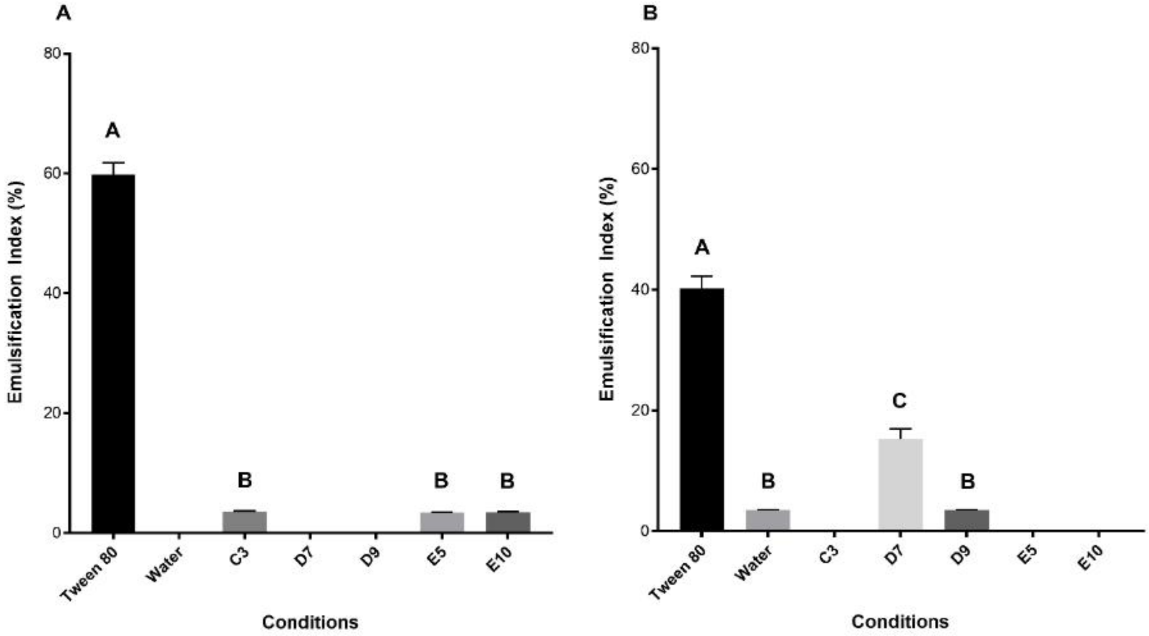
Figure 2. Surface tension of isolated compounds of bacterial strains from the Antarctic.



Medium was used as a negative control and water was used as a positive control. Different letters denote statistically significant differences (p<0,05). Results were expressed as mean ± standard deviation of the mean. (n=3). Source: Elaborated by the authors.

The emulsification index values (E24) are shown in Figure 3. The species C3, E5 and E10, when tested with diesel oil, (Figure 3A) presented a small emulsifying capacity (3.5%) when compared to the control tests. However, when tested with gasoline (Figure 3B), the D7 species showed the highest emulsifying capacity (15%) when compared to the control tests.

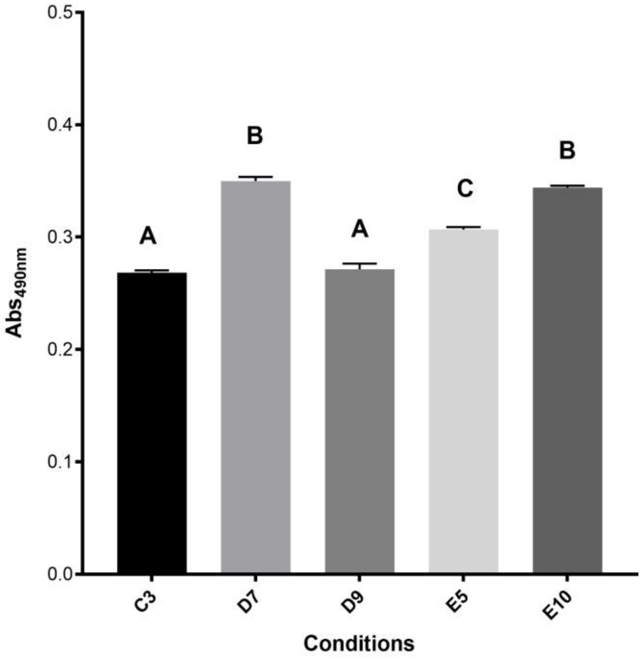
Figure 3. Emulsification index (E24) of isolated compounds of bacterial strains from the Antarctic (C3, D7, D9, E5,and 10) using (A) diesel oil and (B) gasoline.



E24 index is given as a ratio ofthe emulsified layer height (cm) to the total height of the liquid column (cm) expressed as a percentage.Water was used as a negative control and Tween 80 was used as a positive control. Results were expressed as mean ± standard deviation of the mean. (n=3). Different letters denote statistically significant differences (p<0,05). u.a. (arbitrary units). Source: Elaborated by the authors.

In the Figure 4 is shown the Sulfuric Acid/Phenol reaction. All species were reactive to carbohydrates, and species D7 and E10 stood out for the highest absorbance value as 0.350 and 0.344, respectively.

Figure 4. Evaluation of carbohydrates content in the chemical structure of compounds isolated from bacterial strains from the Antarctic.



The test for the presence of carbohydrates was performed by the Duboi test (Duboiset al., 1956) and the samples were analyzed at a wavelength of 490 nm. Results were expressed as mean ± standard deviation of the mean. (n=3). Different letters denote statistically significant differences ($p<0,05$). u.a. (arbitrary units). Source: Elaborated by the authors.

4. Discussion

Several methods can detect biosurfactant after the bioprocess from microorganisms (Carrillo et al., 1996; Satpute et al., 2010b) such as drop collapse (Bodour & Miller-Maier, 1998), oil propagation (Morikawa et al., 2000), index of emulsifier (E24) (Ellaiah et al., 2002) and surface tension (Sidkey et al., 2016). Recently, the screening of biosurfactant production from newly isolated Rhodotorula sp.YBR was carried out with these tests (Derguine-Mecheri et al., 2021). Here, we used the Surface Tension, Drop Collapse, and Emulsification Index tests to identify biosurfactant producing bacterium.

The drop collapse test is a sensitive and easy-to-perform method. Furthermore, it requires a small volume of the sample to test the property of the surfactant compound (Sidkey et al., 2016). Droplet spreading is a consequence of the presence of surface active compounds or tensoative such as biosurfactants (Tugrul & Cansunar, 2005). Drop collapse analyses demonstrated the presence of biosurfactants due to increase the diameter of the droplet applied compared to the distilled water control (Pires et al., 2020). Recently, the screening of biosurfactant production from newly isolated *Rhodotorula* sp. YBR was made with different tests such as drop collapse test (Derguine-Mecheri et al., 2021).

One of the criteria used to identify microorganisms that produce biosurfactants is their ability to reduce the surface tension of water to values below 40 mN/m (Sidkey et al., 2016). This reduction in surface tension is due to the decrease in the cohesion force between the water molecules present on the liquid surface in contact with the air (Felipe and Dias, 2017). In the study conducted by Vasileva-Tonkova & Gesheva (2007), a glycolipid produced by a facultative anaerobic bacterium collected in Antarctic soil (*Pantotea* sp.) reduced the surface tension to values below 37 mN/m, values similar to our study. BS from bacterial strains *P. extremoustralis* (DSM 17835) and *Rodococcus fascians* isolated from an Antarctic environment also demonstrated a significant reduction in surface tension (Tribelli et al., 2012). The bacterium *Pseudomonas fluorescens* BD5 collected from fresh water in the Arctic has demonstrated the ability to reduce the surface tension of water from 72 to 31.5

mN/m (Janek et al., 2012). Interestingly, in the study conducted by Sidkey et al. (2016), the biosurfactant compounds showed a reduction in surface tension to 28.2 mN/m.

Another method to identify BS is the Emulsification Index (E24). Our results are similar to demonstrated in the literature. The screening work carried out by Sidkey et al. (2016) showed results from strains with an emulsification index greater than 35%, values much higher than those found in this study. The BS produced by *Bacillus subtilis* N3-1P strain was 63.11%. Interestingly, in this study the brewery waste was used as the sole carbon source (Moshtagh et al., 2019). A bioemulsifier produced by Mucor circinelloides UCP0001 exhibited 100% emulsification index for canola oil and petroleum (Marques et al., 2020). Recently, production of biosurfactants using *Pseudomonas* spp. from SM fruit pulp as sole substrate showed emulsification index (56.35%) and E24 change with pH (Ejike Ogbonna et al., 2021). Biosurfactants with great properties can stabilize emulsions by at least 50% of the total volume for 24 h (Batista et al., 2006). However, cold-adapted bacterial isolates from Antarctica produced biosurfactants of Emulsion index (E24) 36.4%. These microorganisms are suitable novels for the exploration of hydrocarbon bioremediation in low-temperature environments (Trudgeon et al., 2020). Moreover, the emulsifier characteristics of the biosurfactante have potential application in cosmetic and personal care products (Adu et al., 2020).

The sulfuric acid/phenol reaction was carried out to determine whether the molecule of the surface-active compound has carbohydrate in its chemical structure. This reaction is based on the dehydration of simple sugars, polysaccharides and their derivatives that have free reducing groups by sulfuric acid and subsequent complexation of the products formed with phenol. This complexation causes a change in the color of the solution that is measured in the visible region (Dubois et al., 1956). The biosurfactant isolated from *Rhodotorula mucilaginosa* KUGPP-1 of Antarctic origin also showed activity for carbohydrates using the Sulfuric Acid/Phenol test (Kawahara et al., 2013). The *Pseudomonas extremoustralis* strain from the Antarctic also has carbohydrates present in the structure of the biosurfactant it produces (Tribelli et al., 2012). In the screening performed by Sidkey et al. (2016), the two biosurfactant-producing samples (B13 and B55) were also reactive for sugar when the sulfuric acid/phenol test was performed.

Finally, our study demonstrates a high biotechnological potential to produce biosurfactants, a product with high added value. Moreover, the use of diesel oil waste by bacteria as a carbon source becomes an attractive alternative because it reduces environmental contamination and has a low cost. Surprisingly, the use of diesel waste as a carbon source becomes an attractive alternative to a low-cost substrate, in addition to helping to reduce environmental contamination. A study demonstrated the production of biosurfactant from the bacterium *Corynebacterium aquaticum* from a low-cost substrate (Martins et al., 2018). Interestingly, a study conducted by Lee et al. (2020) showed a bacterium capable of using phenol as the sole source of carbon and energy. Recently, a study demonstrated the combination of fungal strains and biosurfactant-producing bacteria to potentiate the degradation of petroleum hydrocarbons, which could improve bioremediation processes (Atakpa et al., 2022). Petroleum contains many toxic and harmful components with negative impact on the ecological environment and the use of biological methods, as microorganisms, could be an excellent alternative as compared with physical and chemical remediation techniques in addition to produce a bioactive compound as biosurfactants (Sun et al., 2018).

5. Conclusion

This study identified biosurfactants producing bacterial species from soil contaminated by diesel oil in the Antarctic. The biosurfactants produced by bacteria allowed the droplet to spread and acted to reduce surface tension of water, despite not having high emulsifying activity. Also, these molecules test positive for carbohydrates with sulfuric acid. These species have potential application in biotechnological areas such as in bioremediation of environments contaminated by diesel oil and in cosmetic and personal care products. Surprisingly, the use of diesel waste as a carbon source becomes an attractive alternative to a low-cost substrate, in addition to helping to reduce environmental contamination.

The biotechnological potential of the production of biosurfactants by microorganisms collected in Antarctica opens several perspectives for new studies. Future research may look for other microorganisms that produce biosurfactants, in addition to optimizing production by these microorganisms already found.

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References

Adu, S. A., Naughton, P. J., Marchant, R., & Banat, I. M. (2020). Microbial Biosurfactants in Cosmetic and Personal Skincare Pharmaceutical Formulations. *Pharmaceutics, 12*(1099), 1-21. http://dx.doi.org/10.3390/pharmaceutics12111099.

Al-Dhabi, N. A., Esmail, G. A., & Valan Arasu, M. (2020). Enhanced production of biosurfactant from *Bacillus subtilis* strain Al-Dhabi-130 under solid-state fermentation using date molasses from Saudi Arabia for bioremediation of crude-oil-contaminated soils. *International Journal of Environmental Research Public Health, 17*(22), 8446. http://dx.doi.org/10.3390/ijerph17228446.

Anjun, F., Gautam, G., Edgard, G., & Negi, S. (2016). Biosurfactant production through *Bacillus* sp. MTCC 5877 and its multifarious applications in food industry. *Bioresource Technology, 213*, 262–269. http://dx.doi.org/10.1016/j.biortech.2016.02.091.

Atakpa, E. O., Zhou, H., Jiang, L., Ma, Y., Liang, Y., Li, Y., Zhang, D., & Zhang, C. (2022). Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria. *Chemosphere, 290*, 133337. http://dx.doi.org/10.1016/j.chemosphere.2021.133337.

Barros, F. F. C., Quadros C. P., & Pastore, G. M. (2008). Studies of emulsifying properties and stability of the biosurfactant produced by *Bacillus subtilis* in cassava wastewater. *Food Science Technology, 28*(4), 979-985. http://dx.doi.org/10.1590/S0101-20612008000400034.

Batista, S. B., Mounteer, A. H., Amorim, F. R., & Tótola, M. R. (2006). Isolation and characterization of biosurfactant/bioemulsifier-producing bacteria from petroleum contaminated sites. *Bioresource Technology, 97*(6), 868-875. http://dx.doi.org/10.1016/j.biortech.2005.04.020.

Befkadu, A. A., & Chen, Q. (2018). Surfactant-enhanced soil washing for removal of petroleum hydrocarbons from contaminated soils: a review. *Pedosphere, 28*(3), 383–410. http://dx.doi.org/10.1016/S1002-0160(18)60027-X.

Bezza, F. A., & Chirwa, E. M. (2017). Pyrene biodegradation enhancement potential of lipopeptide biosurfactant produced by *Paenibacillus dendritiformis* CN5 strain. *Journal of Hazardous Materials, 321*, 218–227. http://dx.doi.org/10.1016/j.jhazmat.2016.08.035.

Bodour, A. A., & Miller-Maier, R. M. (1998). Application of a modified drop colapse technique for surfactant quantification and screening of biosurfactant-producing microorganisms. *Journal of Microbiological Methods, 32*(3), 273-280. http://dx.doi.org/10.1016/S0167-7012(98)00031-1.

Bodratti, A. M., Sarkar, B., & Alexandridis, P. (2017). Adsorption of poly(ethylene oxide)-containing amphiphilic polymers on solid-liquid interfaces: fundamentals and applications. *Advances in Colloid and Interface Science, 244*, 132-163. http://dx.doi.org/10.1016/j.cis.2016.09.003.

Carrillo, P. G, Mardaraz, C., Pitta-Alvarez, S. I., & Giuliett A. M. (1996). Isolation and selection of biosurfactant producing bacteria. *World Journal of Microbiology and Biotechnology, 12*(1), pp. 82–84.

Cazals, F., Huguenot, D., Crampon, M., Colombano, S., Betelu, S., Galopin, N., Perrault, A., Simonnot, M. O., Ignatiadis, I., & Rossano, S. (2020). Production of biosurfactant using the endemic bacterial community of a PAHs contaminated soil, and its potential use for PAHs remobilization. *Sci. Total Environ., 709*, 136-143. http://dx.doi.org/10.1016/j.scitotenv.2019.136143.

Clements, T., Ndlovu, T., Khan, S., & Khan, W. (2019). Biosurfactants produced by *Serratia* species: classification, biosynthesis, production and application. *Appl Microbiol Biotechnol, 103*(2), 589-602. http://dx.doi.org/10.1007/s00253-018-9520-5.

Christofi, N., & Ivshina, I. B. (2002). Microbial surfactants and their use in field studies of soil remediation. *J Appl Microbiol, 93*(6), 915-929. http://dx.doi.org/10.1046/j.1365-2672.2002.01774.x

Cooper, D. G., & Goldenberg, B. G. (1987). Surface-active agents from two *Bacillus* species. *Appl Environ Microbiol, 53*(2), 224–229. http://dx.doi.org/10.1128/aem.53.2.224-229.1987.

Das, P., Mukherjee, S., Sivapathasekaran, C., & Sen R. (2010). Microbial surfactants of marine origin: potentials and prospects. *Advances in Experimental Medicine and Biology, 672*, 88-101. http://dx.doi.org/10.1007/978-1-4419-5979-9_7.

De Nevers N., & Grahn R. (1991). *Fluid mechanics for chemical engineers*. McGraw-Hill.

Derguine-Mecheri, L., Kebbouche-Gana, S., & Djenane, D. (2021). Biosurfactant production from newly isolated *Rhodotorula* sp. YBR and its great potential in enhanced removal of hydrocarbons from contaminated soils. *World Journal of Microbiology and Biotechnology, 37*(1), 18. http://dx.doi.org/10.1007/s11274-020-02983-3.

Dubois, M., Gills, K. A., Hailton, J. K., Reberes, P. A., & Smit, F. (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry, 28*(3), 350-356. http://dx.doi.org/ 10.1021/ac60111a017.

Dusane, D. H., Pawar, V. S., Nancharaiah, Y. V., Venugopalan, V. P., Kumar, A. R., & Zinjarde, S. S. (2011). Anti-biofilm potential of a glycolipid surfactant produced by a tropical marine strain of *Serratia marcescens. Biofouling, 27*(6), 645-654. http://dx.doi.org/10.1080/08927014.2011.594883.

Ellaiah, P., Prabhakar, T., Sreekanth, M., Taleb, A. T., Raju, P. B., & Saisha, V. (2002). Production of glycolipids containing biosurfactant by *Pseudomonas species. Indian Journal of Experimental Biology, 40*(9), 1083–1086.

Ejike Ogbonna, K., Victor Agu, C., Okonkwo, C. C., Tochukwu Ughamba, K., Akor, J., & Njoku, O.U. (2021). Use of *Spondias mombin* fruit pulp as a substrate for biosurfactant production. *Bioengineered, 12*(1), 1-12. http://dx.doi.org/10.1080/21655979.2020.1853391.

Felipe, L. O., & Dias, S. C. (2017). Surfactantes sintéticos e biossurfactantes: vantagens e desvantagens. *Química Nova na Escola, 39*(3), 228-236.

Grabowski, A., Nercessian, O., Fayolle, F., Blanchet, D., & Jeanthon, C. (2005). Microbial diversity in production waters of a low-temperature biodegraded oil reservoir. *FEMS Microbiology Ecology, 54*(3), 427-443. http://dx.doi.org/10.1016/j.femsec.2005.05.007.

Hajimohammadi, A., Ngo, T., & Kashani, A. (2018). Sustainable one-part geopolymer foams with glass fines versus sand as aggregates. *Construction and Building Materials*, *171*, 223–231. http://dx.doi.org/10.1016/j.conbuildmat.2018.03.120.

Harshada K. (2014). Biosurfactant: a potent antimicrobial agent. *Journal of Microbiology and Experimentation, 1*(5), 1-5. http://dx.doi.org/10.15406/jmen.2014.01.00031.

Helmy, A., De Simoni, M., Guilfoyle, M. R., Carpenter, K. L. H., & Peter, J. H. (2011). Cytokines and innate inflammation in the pathogenesis of human traumatic brain injury. *Progress in Neurobiology, 95*(3), 352–372. http://dx.doi.org/10.1016/j.pneurobio.2011.09.003.

Janek, T., Lukaszewicz, M., & Krasowska, A. (2012). Antiadhesive activity of the biosurfactant pseudofactin II secreted by the Arctic bacterium *Pseudomonas fluorescens* BD5. *BMC Microbiology, 12*(24), 1-9. http://dx.doi.org/10.1186/1471-2180-12-24.

Jimoh, A. A., & Lin, J. (2020). Biosurfactant: A new frontier for greener technology and environmental sustainability. *Ecotoxicology and Environmental Safety Academic Press, 184*, 2-20. http://dx.doi.org/10.1016/j.ecoenv.2019.109607.

Joshi S. J., Geetha S. J., & Desai A. J. (2015). Characterization and application of biosurfactant produced by *Bacillus licheniformis* R2. *Applied Biochemistry and Biotechnology, 177*(2), 346–361. http://dx.doi.org/10.1007/s12010-015-1746-4.

Kawahara, H., Hirai, A., Minabe T., & Obata H. (2013). Stabilization of Astaxanthin by a novel biosurfactant produced by *Rhodotorula mucilaginosa* KUGPP-1. *Biocontrol Science, 18*(1), 21-28. http://dx.doi.org/10.4265/bio.18.21.

Lee, G. L. Y., Zakaria, N. N., Convey, P., Futamata, H., Zulkharnain, A., Suzuki, K., Abdul Khalil, K., Shaharuddin, N. A., Alias, S. A., González-Rocha, G., & Ahmad, S. A. (2020). Statistical optimisation of phenol degradation and pathway identification through whole genome sequencing of the cold-adapted Antarctic bacterium *Rhodococcus* sp. strain AQ5-07. *International Journal of Molecular Science. 21*(24), 9363. http://dx.doi.org/10.3390/ijms21249363.

Luna, J. M., Rufino, R. D., & Sarubbo, L. A. (2016). Biosurfactant from *Candida sphaerica* UCP0995 exhibiting heavy metal remediation properties. *Process Safety and Environmental Protection, 102*, 558–566. http://dx.doi.org/10.1016/j.psep.2016.05.010.

Marques, N. S. A. A., Silva, I. G. S. D., Cavalcanti, D. L., Maia, P. C. S. V., Santos, V. P., Andrade, R. F. S., & Campos-Takaki, G. M. (2020). Eco-Friendly bioemulsifier production by *Mucor circinelloides* ucp0001 isolated from mangrove sediments using renewable substrates for environmental applications. *Biomolecules, 10*(365), 1-15. http://dx.doi.org/doi:10.3390/biom10030365.

Martins, P. C., Bastos, C. G., Granjeiro, P. A., & Martins, V. G. (2018). New lipopeptide produced by *Corynebacterium aquaticum* from a low-cost substrate. *Bioprocess and Biosystem Engineering, 41*(8), 1177-1183. http://dx.doi.org/10.1007/s00449-018-1946-8.

Martins, P. C., & Martins, V. G. (2018). Biosurfactant production from industrial wastes with potential remove of insoluble paint. *International Biodeterioration & Biodegradation, 127*, 10–16. http://dx.doi.org/10.1016/j.ibiod.2017.11.005.

Mnif, I., & Ghribi, D. (2015). Lipopeptides biosurfactants: mean classes and new insights for industrial, biomedical, and environmental applications. *Peptide Science, 104*(3), 129-147. http://dx.doi.org/10.1002/bip.22630.

Morikawa, M., Hirata, Y., & Imanaka, T. (2000). A study on the structure-function relationship of lipopeptide biosurfactants. *Biochimica et Biophysica Acta, 1488*(3), 211–218. http://dx.doi.org/10.1016/S1388-1981(00)00124-4.

Moshtagh, B., Hawboldt, K., & Zhang, B. (2019). Optimization of biosurfactant production by *Bacillus subtilis* N3-1P using the brewery waste as the carbon source. *Environmental Technology, 40*(25), 3371-3380. http://dx.doi.org/10.1080/09593330.2018.1473502.

Nithya, C., Aravindraja, C., & Pandian, S. K. (2010). *Bacillus pumilus* of Palk Bay origin inhibits quorum-sensing-mediated virulence factors in Gram-negative bacteria. *Research in Microbiology, 161*(4), 293-304. http://dx.doi.org/0.1016/j.resmic.2010.03.002.

Nitschke, M., & Silva, S. S. (2018). Recent food applications of microbial surfactants. *Critical Reviews in Food Science and Nutrition, 58*(4), 631–638. http://dx.doi.org/10.1080/10408398.2016.1208635.

Patel, S., Homaei, A., Patil, S., & Daverey, A. (2019). Microbial biosurfactants for oil spill remediation: pitfalls and potentials. *Applied Microbiology and Biotechnology*, *103*(1), 27-37. http://dx.doi.org/10.1007/s00253-018-9434-2.

Phulpoto, I. A., Yu, Z., Hu, B., Wang, Y., Ndayisenga, F., Li, J., Liang, H., & Qazi, M. A. (2020). Production and characterization of surfactin-like biosurfactant produced by novel strain *Bacillus nealsonii* S2MT and it's potential for oil contaminated soil remediation. *Microbial Cell Factories, 19*(1), 145. http://dx.doi.org/10.1186/s12934-020-01402-4.

Pires, M. E. E., Parreira, A. G., Silva, T. N. L., Colares, H. C., da Silva, J. A., de Magalhães, J. T., Galdino, A. S., Gonçalves, D. B., Granjeiro, J. M., & Granjeiro, P. A. (2020). Recent patents on impact of lipopeptide on the biofilm formation onto titanium and stainless steel surfaces. *Recent Patents on Biotechnology, 14*(1), 49-62. http://dx.doi.org/10.2174/1872208313666190822150323.

Satpute, S. K., Banat, I. M., Dhakephalkar, P. K., Banpurkar, A. G., & Chopade, B. A. (2010). Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. *Biotechnology Advances, 28*(4), 436-450. http://dx.doi.org/10.1016/j.biotechadv.2010.02.006.

Satpute, S. K., Banpurkar, A. G., Dhakephalkar, P. K., Banat, I. M., & Chopade, B. A. (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. *Critical Reviews in Biotechnology, 30*(2), 127–144. http://dx.doi.org/10.3109/07388550903427280.

Sharma, P., & Sharma, N. (2020). Microbial biosurfactants-an ecofriendly boon to industries for green revolution. *Recent Patents on Biotechnology, 14*(3), 169-183. http://dx.doi.org/10.2174/1872208313666191212094628.

Sidkey, N. M., Mohamed, H. F., & Elkhouly, H. I. (2016). Evaluation of different screening methods for biosurfactant producers isolated from contaminated Egyptian samples grown on industrial olive oil processing waste. *British Microbiology Research Journal, 17*(4), 1-19. http://dx.doi.org/10.9734/BMRJ/2016/28437.

Sun, W., Cao, W., Jiang, M., Saren, G. G., Liu, J., Cao, J., Ali, I., Yu, X., Peng, C., & Naz, I. (2018). Isolation and characterization of biosurfactant- producing and diesel oil degrading *Pseudomonas* sp. CQ2 from Changqing oil field, China. *Royal Society of Chemistry Advanced, 8*, 39710–39720. http://dx.doi.org/10.1039/c8ra07721e.

Suthar H., & Nerurkar A. (2016). Characterization of biosurfactant produced by *Bacillus licheniformis* TT42 having potential for enhanced oil recovery. *Applied Biochemistry and Biotechnology, 180*(2), 248-260. http://dx.doi.org/10.1007/s12010-016-2096-6.

Thomas, G. E, Brant, J. L., Campo, P., Clark, D. R., Coulon, F., Gregson, B. H., McGenity, T. J., & McKew, B. A. (2021). Effects of dispersants and biosurfactants on crude-oil biodegradation and bacterial community succession. *Microorganisms*, *9*(6), 1-23. http://dx.doi.org/10.3390/microorganisms9061200.

Tribelli, P. M., Di Martino, C., López, N. I., & Iustman, L. J. R. (2012). Biofilm lifestyle enhances diesel bioremediation and biosurfactant production in the Antarctic polyhydroxyal kanoate producer *Pseudomonas extremaustralis. Biodegradation, 23*(5), 645–651. http://dx.doi.org/10.1007/s10532-012-9540-2.

Trudgeon, B., Dieser, M., Balasubramanian, N., Messmer, M., & Foreman, C. M. (2020). Low-temperature biosurfactants from polar microbes. *Microorganisms, 8*(8), 1-14. http://dx.doi.org/10.3390/microorganisms8081183.

Tugrul, T., & Cansunar, E. (2005). Detecting surfactant-producing microorganisms by the drop-collapse test. *World Journal of Microbiology and Biotechnology, 21*(6), 851-853. http://dx.doi.org/10.1007/s11274-004-5958-y.

Varvaresou, A., & Iakovou, K. (2015). Biosurfactants in cosmetics and biopharmaceuticals. *Letters in Applied Microbiology, 61*(3), 214-223. http://dx.doi.org/10.1111/lam.12440.

Vasileva-Tonkova, E., & Gesheva, V. (2007). Biosurfactant production by Antarctic facultative anaerobe *Pantoea* sp. during growth on hydrocarbons. *Current microbiology, 54*(2), 136–141. http://dx.doi.org/10.1007/s00284-006-0345-6.

Yong, L., Huifeng, W., Xiaoting, L., & Jinchang, L. (2015). Heavy metal contamination of agricultural soils in Taiyuan, China. *Pedosphere, 25*(6), 901–909. http://dx.doi.org/10.1016/S1002-0160(15)30070-9.