Isolation of Bacterial Strains that Produce Biosurfactant for Industrial and Medical Use

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Abstract

The aim of this study is to treat and value petroleum waste by determining if specific microbes can degrade the hydrocarbons (with emulsifying potential) in samples polluted by this waste. On an additional topic, studies on the characteristics and isolation of hydrocarbonoclastic soil bacteria are identified oil quagmire obtained from containers for storage and abandoned refineries for oil. To do this, a wide range of bacterial species have been isolated from oil quagmire samples polluted by hydrocarbures. Isolation permitted us to purify 88 isolates, and an additional evaluation of their emulsifying ability using the E24 test permitted us to choose seven of the best-performing isolates with significant emulsifying capacity.

Keywords: Oil Rejects, Hydrocarbonoclastic Bacteria, Biosurfactants, Emulsification Capacity, Hydrocarbures

1. Introduction

Biosurfactants are used in industry in various ways due to their amphiphilic properties: as adhesive agents, flocculants, wetting agents, foaming agents, emulsifiers, dispersants or as detergents. They are mainly used by the cosmetic industry, particularly for their role as emulsifier, and by the petroleum industry. By increasing the apparent solubility of petroleum compounds and decreasing the interfacial tension between water and crude oil, the mobility of the latter is increased, which allows an increase in the volumes recovered (Cazals, 2020). Petroleum hydrocarbons are a group of widely used products with

many uses across the world. Algeria is one of the main producers and exporters of energy from fossil fuels such as gas and oil (Rammade, 2007). This extensive use could result pollution caused by hydrocarbons both the soil and water around the petroleum industry's facilities and machinery (Norini, 2007). So, physical and chemical elimination methods include usage limits due to their cost and additional impacts on the environment. However, Natural surfactants or biosurfactants are amphiphilic derived from microorganisms, biosurfactants are privileged molecules due to their innate biocompatibility, biodegradability, less toxicity which make them suitable for various applications, especially in the biomedical field. Because of these advantages and the structural diversity, biosurfactants have emerged as potential antitumor, antibacterial, antiviral, antiadhesive agents and also constitute a critical component of drug delivery cargoes (Joshi and Jadhao).

However, the biological approach is currently the most used. In fact, some bacteria exhibit the ability to produce biosurfactants, which are biomolecules having functional characteristics which permit them to utilize carbohydrates as sources of energy and carbon. Several uses are available for these biosurfactants that including biological remediation, petroleum extraction, cosmetics uses, detergents and emulsifier use in the food industries. Indeed, many types of *Bacillus*, *Rhodococcus*, *Serratia*, and *Pseudomonas* are known to produce powerful biosurfactants. Particularly able to produce biosurfactants, are yeast cells like *Candida* and certain kinds of fungi such *Aspergillus*.

The emulsifier known as biosurfactants are produced by a number of microbes. Their nature and tension ability are greatly influenced by the type of organisms (bacteria, yeasts, or fungi that is utilized, the strain which is tested, and the nutritional substrate that is available for their cell growth. Currently, the list of acknowledged biosurfactants include glycolipids, lipopeptides, phospholipids, neutral lipids, fatty acids, and lipopolysaccharides.

In this study, we isolated a wide range of bacteria utilizing samples collected from multiple hydrocarbon-contaminated locations. These purified isolates undertook an emulsifying assay to determine the strongest bacterium capable to generate a more significant quantity of biosurfactant. The selected isolates were subsequently examined for microbial identifying assays after being employed for the treatment of a hydrocarbon reject samples.

2. Materials and Methods

2.1. Source of Organisms and Samples

We employed oil quagmire from an Algerian petroleum industry, and the samples were taken between April 4 and May 11, 2022, at the southern Algerian location. The samples were immediately transported at ambient temperature to the laboratory and stored at 4º C until analyses. Samples were treated within 24 h after collection.

2.2. Physical-Chemical Analysis of Samples

We determined some parameters, including the total hydrocarbures rate (TPH), the amount of asphalt and the paraffin content, as well as the levels of Hg and Lead. Salinity and levels of pH have been determined **(**American Public Health Association, 2005).

2.3. Bacterial Isolation, Media and Growth Conditions

Cultivation of the bacteria species requires a stage of reactivation and enrichment**.** A Nutrient agar medium for aerobic microorganisms has been used, it for enrichment, isolation, and for physiological identification tests, containing (per liter); 5 g of NaCl, 5 g of peptone and 3 g of yeast extract, adjusted to pH 7 with HCl. Enrichments are made in 250 ml Erlens Meyer autoclaved at 120° C for 20 minutes, filled with 100 ml of culture medium then inoculated 1/10 with the oil quagmire sample and the various dilutions prepared. Incubation of the cultures takes place in a shaker incubator at 40° C with a stirring speed of 120 rpm.

So, dilutions of 10^{-1} to 10^{-4} were made on the same agar medium solidified with 20 g of agar. After 2-3 days of incubation at 40°C, the colonies were picked and sub-cultured several times to obtain a pure culture.

2.4. Screening for Biosurfactant Production

The resulting supernatant was subjected to the E24 test determinations as described by Bodour and Miller-Maier (1998). After growing in standard for seven days in an orbital shaker at 160 rpm and 40° C. The pure isolate was grown on Nutrient liquid media for 48 h at 40° C with constant shaking of 120 rpm. The broth was centrifuged at 5000 rpm for 20 min. cells were removed by centrifugation at 12,000 × g for 5 min at room temperature. 2 mL of the cell-free supernatant were mixed with 2 mL diesel in a test tube (100 mm × 15 mm). This mixture was shaken for 3 min and then left to stand. Relative emulsion volume (EV, %) and stability (ES, %) were measured in intervals up to 48 h using the following equations.

$$\text{EV, \%} = \frac{\text{EV; \% Emulsion Height (mm)} \times \text{Cross-section Area } (mm^2)}{\text{Total Liquid Volume } (mm^3)} \times 100$$

$$\text{\% ES} = \frac{\text{\% EV; \% at time t; h}}{\text{EV; \% at 0 h}} \times 100$$

Emulsions formed by the isolates were compared to those formed by a 1% (w/v) solution of the synthetic surfactant sodium dodecyl sulphate (SDS) in deionised water.

2.5. Extraction of the biosurfactant

The culture broth was centrifuged in a refrigerated centrifuge at 10,000 rpm for 20 min at 4° C. The supernatant obtained was filtered through 0.2 µm filter, the pH of the filtered supernatant was adjusted to 2 using 1M HCl, then subjected to acid precipitation at 4° C overnight and followed by liquid–liquid extraction method. The supernatant obtained was extracted thrice with an equal volume of ethyl acetate and methanol (2/1, v/v), shaking vigorously and allowing the two layers to separate. The biosurfactant was concentrated using a rotary evaporator at 40° C to remove the solvent. Biosurfactant yield and biomass were expressed in g L−1(Derguine-Mecheri et al. 2018). The extract material was analysed by thin-layer chromatography. Plates for thin layer chromatography (TLC) (Silica gel 60A), were washed with chloroform/methanol (1:1, v/v) and activated at 120° C before use. Peptidic and glucidic components were separated in solvent S1 (chloroform-methanol-acetic acid, 80:18:2 by volume). The peptidic components were visualized by staining them with ninhydrin (5 mg of ninhydrin in a 50 ml butanol-50 ml acetone mixture) and heating them at 100° C for 5 min. Sugar compounds were located

by charring at 110° C for 5 min after spraying anthrone reagent. The lipid components were detected as brown spots on the plate after spraying with chromosulfuric acid.

3. Results and Discussion

3.1. Physical-Chemical Analysis of the Samples

Drilling fluid, sometimes referred to as drilling mud in geotechnical engineering, is mostly contaminated with heavy metals and hydrocarbons that primarily come from fluid. So, Table 1 displays the results of analyses of several physical-chemical variables associated with our samples. Compared to the other previously mentioned testing, the NB1 sample has an extremely high hydrocarbures amount of 94857 (mg/g). Furthermore, the remaining samples have low hydrocarbures rates. Additionally, the samples indicate an average content in their respective variant among 1 and 3.

Table 1: Results of Some Physical-Chemical Analyses of the Samples

Sample	NB1	AB	NB	AB FOND
TPH (mg/g)	94857	11.2	44	470.09
Salinity (PSU)	3.05	2.71	1.3	1.374
pH	4.88	6.62	7.14	6.043
Hg (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1
Lead (µg/L)	243.983	64.28	58.719	139.994

However, salinity decreases the number of microorganisms in the biotope and inhibits the processes of humify and mineralize organic matter, such as nitrification and emission of CO_2. As a result, high levels of salinity constitute a natural barrier to biological degradation (Blifert et Perraud, 2004). In contrast, nearly all samples have a pH that is near neutral. The pH has significant effects on the growth of bacteria. It needs to be mounted among 5 and 9, preferably within 7 limits. Acid pH could promote the dissolution of heavy metals that are highly toxic to microbes (Blifert et Perraud, 2004).

3.2. Selecting Microorganisms that Produce Biosurfactants

The ability of the various isolates to produce biosurfactants was assessed through a cribbage using the E24 test of emulsification method. A few isolated strains have demonstrated elevated E24 values, reaching as high as 40%, whereas other strains have showed low levels (less than 10%). According to Mukesh et al. (2012), the emulsification index is dependent regarding the variety of the biosurfactant and producing microorganisms According to Previous research (Benincasa et Accorsini, 2008; Lovaglio et al., 2011) demonstrated emulsifying indices of 50% and 58%, respectively. Desai and Patel, 1997. Pereira et al. (2013) obtained a strain that corresponded to the B. subtilis species and displayed a 52.7% n-hexadecane emulsifying power. Additionally, the seven (7) isolates shown in Table 2 were selected as performing because of their capacity to produce biosurfactant. The results of the E24 test of emulsifying that was carried out on 88 separated strains based on different samples.

Table 2: Selecting Microorganisms that Produce Biosurfactants

Isolate	E 24% Test	
	At Time 0 h	After 24 h
NB1 7	59	50
NB2 5	67.31	62
NB2 11	78.57	78
BE	50	45
NB3 2	46	35
NB3 2	46	35
NB3 5	28.8	4

Indeed, the highest percentage (78%) was reported by the sample NB2 11. The other samples, NB2 5, BE, NB1 7, and NB3 2, had less significant percentages of 67.31%, 50%, and 46%, respectively. These results can be compared to those obtained by Guregouri (2010), who discovered that the most efficient strain of bacteria to produce biosurfactants had an index of E24 equal 63% belonging to the Gram-negative bacteria. According to Rodríguez-Rodríguez et al. (2012), an E24 > 50% indicates that the microorganism may produce biosurfactants. However, it is important to consider the ideal circumstances for the maximum productivity of biosurfactants.

3.3. Chromatographic Behaviour

A few criteria need to be achieved by the biosurfactant to be effective for these techniques of separating to be successful for the purpose of the centrifugation. The insoluble biosurfactant becomes precipitate due to the centrifugation force, and for acid precipitation, the biosurfactant becomes insoluble at acidic pH values for the purpose of solvent extraction.

Due at the hydrophobic compound, utilizing organic solvents allowing the biosurfactants to dissolve. After extracting and unidimensional chromatography on a thin layer. Compound characterization with TLC is also possible and is similar to reaction monitoring. However, rather than spotting with starting material and reaction mixture, it is with an unknown and a known compound.

Table 3: Rf Values Obtained with TLC

		Strain NB2-11	
		Numbers of Spot	Rf Values
Components	Glucidic	3	0.13 ; 0.17 ; 0.47
	Peptidic	4	0.21 ; 0.27 0.31 ; 0.36
	Lipidic	0	-

The obtained results confirm that the bacterial strain NB2 II is producing extracellular iochemicals as evidenced by the presence of sugars and peptidic compounds. Table 3 indicates that the biosurfactants

produced by this strain contain of both peptidic and glucidic components; these are most. This could be a peptidoglycolipid thus, such as glycoproteins.

Conclusion

Tensioactifs have a physiological function by promoting the growth of microorganisms on hydrophobic substrate through decreasing the interfacial tension among the substrate and water, allowing the latter faster to access. Usually, the bacteria that are widely used in the synthesis of biosurfactants come from soil that has been contaminated with hydrophobic molecules like HAP. The objective of this study was to isolate that proved to be both efficient at producing biosurfactant and high-performing. As a result, it was possible to isolate various strains using oil quagmire samples obtained across different Algerian regions. 88 isolated strains were subjected to a screening procedure using the E24 test with the objective to determine which of them were particularly effective in producing biosurfactants. Furthermore, the E24 test allowed us to preselect seven providers of biosurfactant. Also, the biosurfactants generated through these bacteria stains may be used for several biotechnological, industrial and medical uses.

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