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RESEARCH ARTICLE

ISOLATION OF BACTERIAL BIOSURFACTANTS FROM LOCAL BACTERIAL STRAINS

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ABSTRACT

Soil samples were collected from oil polluted station from Majnoon oil fieldat a latitude, 30°53'38.36"N and longitude of 47°32'26.18"E, Basrahcity, the samples were cultivated on Minimal salt medium contain crude oil as a sole source of carbon, four types of growing oil degraded bacteria identified according to their morphological and biochemical profiles *as :Aeromonashydrophila, Bacillus subtilis, Pseudomonas aeruginosa* and *Pseudomonas fluorescens* after using specific cultured media. The ability of defined species for production of biosurfactants have been checked by several methods, drops collapse test, blood haemolysis test and oil spreading test. All these bacterial specieswere determinate as biosurfactants producing species, the best results detected with *Pseudomonas fluorescens,* biosurfactants have been extracted and kept for further studies.

INTRODUCTION

Biosurfactants are compounds that produce surface-active and emulsifying activities and are themselves producedby microorganisms, such as bacteria, yeast and fungi. For example, *Bacillus subtilis* is known to produce surfactin (Pornsunthorntawee *et al*., 2008) while *Pseudomonas aeruginosa* has been reported to produce the biosurfact antrhamnolipid (Cameotra and Makkar 2004). Biosurfactants are amphiphilic, consisting of two parts, a polar (hydrophilic) moiety and a non-polar (hydrophobic) group. The hydrophilic group consists of mono-, oligo-, or polysaccharides, peptides or proteins while the hydrophobic moiety usually contains saturated, unsaturated and hydroxylated fatty acids or fatty alcohols (Pacwa-Plociniczak *et al.,* 2011). Biosurfactants play a number of roles including increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, binding of heavy metals, quorum sensing and biofilm formation (Rodrigues *et al.,* 2006). In the current study, Production and extraction of biosurfactant was carried out using *Pseudomonas aeruginosa, Pseudomonas fluorescence, Aeromomashydrophila and Bacillus subtilis*, The extraction and emulsificationpotency of the surfactants have been performed, The surfactants contents were assayed by many tests.

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MATERIALS AND METHODS

Soil Sampling

Soil samples have been collected from oil polluted stations from Majnoon oil field, Basra, southern of Iraq (Figure 1), Soil samples (150 mg) were collected in plastic bags until returned to the laboratory.

Isolation of bacterial species

One gram of oil polluted soil were diluted with sterile D.W to 10^{-3} then cultivated in a conical flask containing 100 ml of mineral salts medium (MSM): KCl,0.3gm. K2HPO4,1.0gm. KH2PO4, 0.5gm. FeSO4.7H2O,0.01gm. NaCl,30.0gm. MnSO4.7H2O, 0.5gm. CaCl2, 0.2gm. D.W, 1000ml (Fujisawa and Murakami, 1980). With 0.1 ml of crude oil (provided from Majnoon oil field) for 7 days at 22°C, 0.1 ml growth culture diluted several times in 9 ml of normal saline and cultivateon Pseudomonas agar base (Himedia) to isolate *Pseuromonas* species, Ampicillin Dextrin agar (Himedia) to isolate *Aeromonashydrophila* and Luria-Bertani agar (Himedia) to isolate *Bacillus subtilis* by spreading method. The presumptive colonies were subjected to the gramstain, oxidase, catalase, nitratereduction, motility, gelatin liquefaction, endospore, endole, methyl red, Voges Proskauer, citrateutilization, growth at 4°C, growth at 41°C, fluorescence and H_2S tests (Holt *et al.*, 1994).

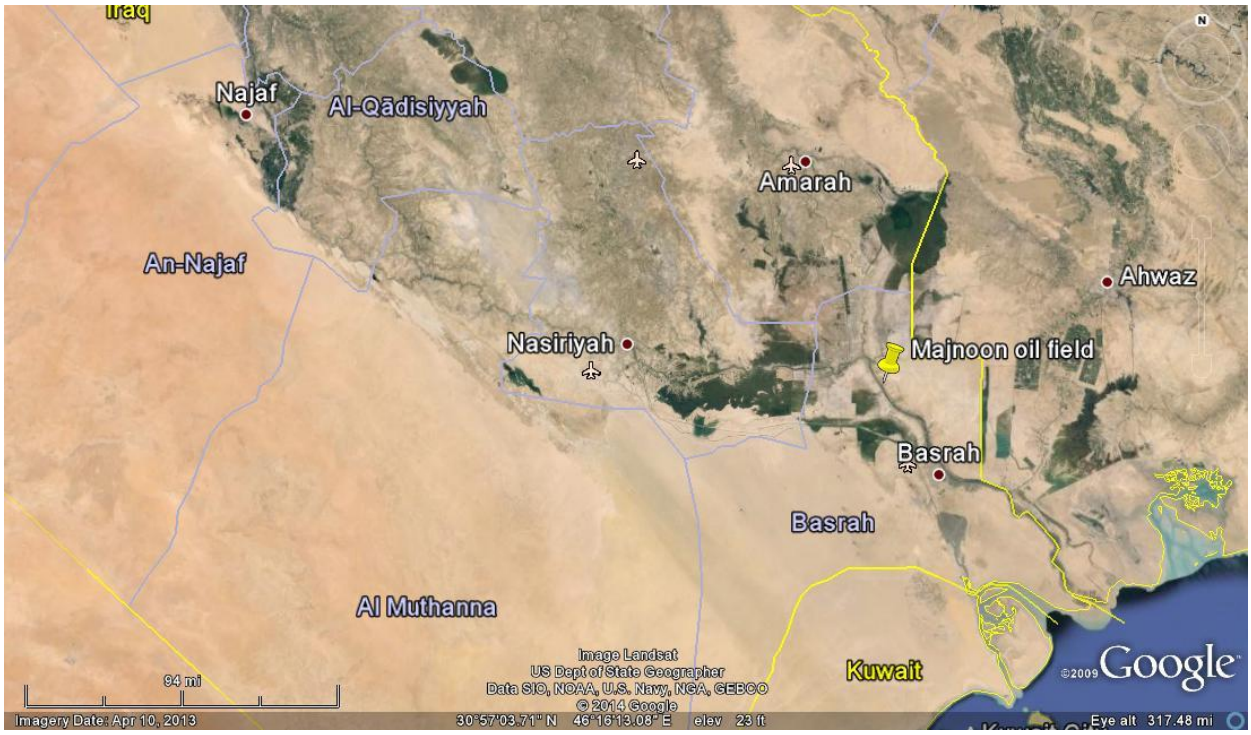

Fig. . Sampling map

Biosurfactants detection methods

Blood hemolytic activity

Biosurfactant producing bacteria have ahemolytic activity on Blood Agarmedium and this test was used to decrease and eliminate in appropriate samples.

The isolated bacteria were cultivate don Blood Agarandincubated at 30°C for 72h (Bicca *et al*., 1999). The bacteria with ahemolytic activity were detected as biosurfactants producer.

Oil spreading method

30ml of distilled water was taken in the petri plates, then 1mlofcrude oil was added to plate containing distilled water (in the centre) then add15µlof the supernatant of the each isolate to the centre, the oil will displace by biosurfact antproducing organism (Vanmathy Selvi and Nithya, 2014)

Drop-collapse method

In the drop collapsing test, a drop of a cell suspension is placed on an oil-coated surface. Drops containing biosurfactants collapse, whereas non-surfactant containing drops remains stable. In this study, distilled water used as a control. Soybean oil was used as a coating oil in the drop- collapse method. glass syringe (Hamilton microsyringe) was used to add the biosurfactants to the polystyrene micro well plate (12·8cm), the plate contains wells with 8mm internal diameter and 0.30 mm depth. Each well was coated with Soybean oil and left at room temperature for24 h. After that, by the syringe at an angle of 45, 20 µl of supernatant and 20 µl of each control was added to each well. After 1 min, the drops were examined visually. The syringe was rinsed three times with distilled water, three times with alcohol and three times with ether between each measurement (Tugba Tugrul and Emir Cansunar, 2005).

Extraction of Biosurfactan

The production was carried out with the optimized condition for 48 hours and the bacterial cells were removed by centrifugation at 10.000 RPM for 20 min under cooling conditions. In order to precipitate the lipids and proteins, to bring the final pH of 2.0, concentrated HCl was added to the supernatant and kept for overnight at 4°C. H. Yin *et al*. (2008). Gray, white precipitation result was collected by centrifugation at 10.000 RPM for 20 min at 4°C, then 10mL of chloroform methanol (2 : 1v/v) was added to precipitate pellet for further extraction of biosurfactant compounds, and incubated at 30°C for 15 minutes with 250 RPM in a rotatory shaker. The content was centrifugation at10.000 RPM for 20 min under cooling conditions and the supernatant was evaporated by air drying. The remaining residue was dispensed in sodium phosphate buffer (pH 7.0) and stored at 4°C.C. R. Suresh Chander *et al*. (2012).

RESULTS AND DISCUSSION

Four bacterial species were identified according to their morphological and biochemical profile, these species are oil degrading bacteria because their ability to grow in MSM containing a hydrocarbon compounds (crude oil) as a sole source of carbon, the capability of native bacteria to degrading crude oil hydrocarbons in crude oil contaminated soils were ensure before by many scientists (Emtiazi and Shakarami, 2004; Okerentugba, 2003).

Fig. . Up right: *Bacillus subtilis* down right: *Pseudomonas aeruginosa*

Up left: *Pseudomonas fluorescence* down left: *Aeromomashydrophila*

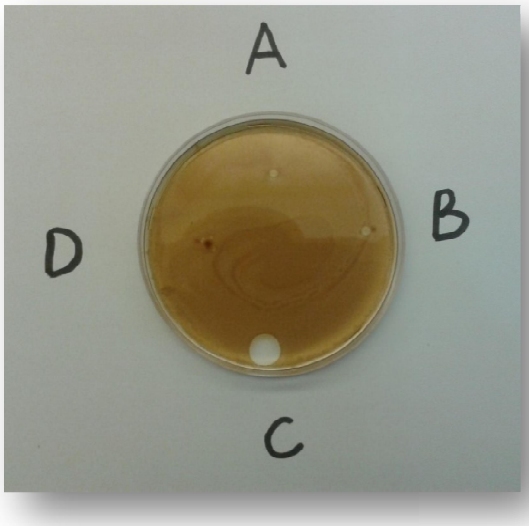

Fig. . Oil, spreading method, C: *Pseudomonas fluorescence*

Fig. . Drops collapse test, Right:D.W,Left:*Pseudomonas fluorescence*

The isolated strains were tested for haemolytic activity which is regarded as indicative for production of biosurfactant and used as a method for bacterial screening (Vandana, 2012). The isolated bacteria have the ability for Beta blood haemolysis and that appear clear in *Pseudomonas fluorescens* (Figure 2) Compared with control. The diameter of water displaces with supernatant in oil spreading test deferent in these four bacteria species, *Pseudomonas fluorescens* show larger diameter (13mm) than the others as shown in (Figure 3). In the drop collapse test *Pseudomonas fluorescens* gives a positive test (Figure 4) when the cell suspension added to the oil coated wells because the addition of cell suspension containing surfactants lowering the surface and interfacial tension while distilled water drop did not collapse and appeared like stable drops. The extracted biosurfactansts have been kept for further studies

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