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ISOLATION AND IDENTIFICATION OF BIOSURFACTANTS PRODUCING BACTERIA FROM CRUDE OIL CONTAMINATED SOIL IN THE RUMAILA OIL FIELD

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Abstract

The present study includes isolation and identification of two bacterial species from soil contaminated with crude oil in the Rumaila oil field at Basrah Governorate capable of producing biosurfactant, i.e. *Kocuria kristinae*, *Rhodococcus* sp. The bacterial species *Kocuria kristinae* that was isolated for the first time in Iraq was highly effective in emulsification index test as the ratio 66% and had a clearing zone of 8 cm in oil spreading test followed by *Rhodococcus* sp. as the ratio 50% in emulsification index test and 7.5 cm in oil spreading test. We conclude that is a possibility of using efficient species in bioremediation of polluted soils with crude oil and its derivatives.

Key words: Biosurfactants, Drop collapse test, Contaminated Soil, Emulsification index test, oil spreading test.

1. INTRODUCTION

Biosurfactants are surface active compounds that are synthesized by microbes like bacteria, fungi and yeast.¹ They are very interesting because their advantages such as biodegradability, low toxicity and ecological acceptability and they possess a wide range of industrial application such, health care, oil and food processing industries, foaming agents, spreading agents, wetting agents and bioremediation.^{1,2,3} Contaminated soil with oil contains large amount of hydrocarbons so

microorganisms exhibit emulsifying activity by producing biosurfactants and utilize the hydrocarbons as substrate often mineralizing them or converting them into harmless products.⁴ The objective of the present study is to isolate biosurfactants producing bacteria from oil contaminated soils in Al-Rumaila oil fields at Basrah Governorate-Iraq.

2. MATERIALS AND METHODS

2.1. COLLECTION OF SAMPLES

Crude oil contaminated soil samples were collected from a contaminated area in the Rumaila oil field in Basrah Governorate during the period between October and November 2017. Samples were kept in sterile plastic vials, and brought to the laboratory.

2.2. METHOD OF ISOLATION BACTERIA

One gram of soil from each sample was added to 100 ml of Minimal Salt Medium (MSM) in conical flasks and incubated at 37 C⁰ for one week in shaker incubator.⁵ After that a drop was taken from the turbid culture and spread over the nutrient agar plates. The plates were incubated at 37 C⁰ for 24 hour. Morphologically different colonies observed on the plates were sub cultured on a nutrient agar plates to obtain pure culture of bacteria for further identification.

2.3. IDENTIFICATION OF ISOLATES

Pure bacterial isolates were identified using gram stain and Vitek 2 compact system where GP kit was used .

2.4. SCREENING OF BACTERIAL ISOLATES FOR BIOSURFACANTS PRODUCTION

The isolates were inoculated into 10 ml of broth medium each and incubated at 37C⁰ for 72 hour . The supernatant was collected and the cells was discarded . The supernatant was used for the various biosurfactant screening tests.⁴

2.4.1.OIL SPREADING TEST

Ten microliter of crude oil was added to the surface of 30 ml of distilled water into a petri dish. A drop of culture supernatant of each isolate was added on the centre of the oil layer. The crude oil will be displaced and a clearing zone will be formed if biosurfactant is present in the supernatant .⁶ Diesel oil was also used in this test . Diameter of clear zone was measured and recorded.

2.4.2.DROP COLLAPSE TEST

A drop of the culture supernatant was placed on a crude oil coated glass slide and observed after one minute . If the culture supernatant contains a biosurfactant , the drop will spread or collapse.⁷

2.4.3.THE EMULSIFICATION INDEX TEST

The test was carried out by adding 2 ml of diesel oil to 2ml of the culture supernatant in test vial and the mixture was vortexed at high speed for 2 minutes .The mixture was then left for 24 hour . The emulsion index E₂₄ was calculated as the ratio of the height of the emulsion layer and the total height of liquid .⁸

3. RESULTS

3.1. ISOLATION AND IDENTIFICATION OF THE ISOLATES

The isolated bacterial colonies were gram positive.They were identified using vitek 2 compact system and morphological and microscopic analysis . (Table 1) .

Table1 : Isolated bacterial species and its method of Identification

Bacterial species	Identification method
<i>Kocuria kristinae</i>	Vitek 2 GP card
<i>Rhodococcus</i> sp.	Morphological and microscopic analysis

3.2. SCREENING FOR BIOSURFACANTS PRODUCTION

Three methods were applied to be sure biosurfactant production in the present study; oil spreading test, drop collapse test and emulsification index test. The results of oil spreading test were shown in (Table2) and (Table3). *Kocuria kristinae* showed highest displacement activity with the largest displacement diameter of crude oil and diesel oil followed by *Rhodococcus* sp.

Table 2: Oil spreading test using crude oil for bacterial species supernatant

Bacterial species	Oil spreading score	Diameter of halo zone
<i>Kocuria kristinae</i>	++++	8 cm
<i>Rhodococcus</i> sp.	++	4.5 cm

Table 3: Oil spreading test using diesel oil for bacterial species supernatant

Bacterial species	Oil spreading score	Diameter of halo zone
<i>Kocuria kristinae</i>	++++	8
<i>Rhodococcus</i> sp.	+++	7.5

According to the results of the drop collapse test, the two bacterial species were positive for the test (Table 4).

Table 4: Drop collapse test using crude oil for bacterial species supernatant

Bacterial species	Drop collapse test
<i>Kocuria kristinae</i>	+
<i>Rhodococcus</i> sp.	+

As for the results of the emulsification index test using diesel oil, the bacterial species *Kocuria kristinae* showed the highest value in this test (66%) followed by *Rhodococcus* sp. (50%) (Table 5).

Table 5 : The emulsification index test (E24%) using diesel oil for supernatant bacterial species

Bacterial species	Emulsification index test
<i>Kocuria kristinae</i>	66%
<i>Rhodococcus</i> sp.	50%

4. DISCUSSION

Biosurfactant producing bacteria were isolated from soil contaminated with crude oil from Al Rumaila oil field which is one of the main fields and it is located in Basrah Governorate, Southern of Iraq. It includes several oil wells. In the present study the bacterial isolates were collected from Al Rumaila oil field and they were belonged to two species; *Kocuria kristinae* and *Rhodococcus* sp. Most of screening of biosurfactant production methods based on interfacial activity. The three screening methods; oil spreading test, drop collapse test and emulsification index were carried out in this study and revealed that the two bacterial species were positive for biosurfactant production.⁴ Oil spreading test was carried out in the study to ascertain if the isolates can displace oil at the surface of water showed that the result gotten in the study was accordance with the findings of Adebajo.¹ Therefore, it is a reliable test for detection of biosurfactant production by microorganisms even activity and quantity of biosurfactant are low.⁹ The present study revealed that *Kocuria kristinae* showed better potential in the entire screening tests for biosurfactant production followed by *Rhodococcus* sp. as shown in Tables 2,3 and 5. No previous study mention to the role of *Kocuria kristinae* in oil degradation and biosurfactant production. While another species is

Kocuria rosea was previously isolated from pesticide contaminated soils.¹⁰

5. CONCLUSION

Screening of biosurfactant producing bacteria from oil-contaminated soil in Al-Rumeila oil field was revealed that the possibility of using the two bacterial species *Kocuria kristinae* and *Rhodococcus* sp. in the bioremediation of soils contaminated with crude oil and its derivatives .

6. REFERENCES

- [1] Adebajo ,S.O. ; Akintokun ,A.K. and Balogun ,S.A. (2017) . Screening and Characterization of Biosurfactant Producing Bacteria from Soil Samples in Ogun-State,Nigeria . *Microbiol . Res. Int. J.*,18(2) :1-10 .
- [2] Budsabun ,T. (2015) .Isolation of Biosurfactant Producing Bacteria from Petroleum Contaminated Terrestrial Sample that Collected in Bangkok ,Thailand .*Proce .Soci. Behav. Sci .*, 197 : 1363-1366 .
- [3] Mahalingam , P.U. and Sampath , N. (2014). Isolation , Characterization and Identification of Bacterial Bio- surfactant *Europ . J. Exp. Biol .*, 4(6): 59-64.
- [4] Femi-Ola ,T.O.; Oluwole ,O.A.; Olowomofe ,T.O. and Yakubu , H.(2015). Isolation and Screening of Biosurfactant Producing Bacteria from Soil Contaminated with Domestic Waste Water .*Brit. J. Environ . Sci .*, 3(1) : 58-63 .
- [5] Patowary, K.;Patowary, R.; Kalita, M.C and Deka,s.(2017) . Characterization of Biosurfactant Produced during Degradation of Hydrocarbons Using Crude Oil as Sole Source of Carbon . *Front . Microbiol .*, 8: 279 .
- [6] Morikawa,M.; Hirata,Y. and Imanaka ,T. (2000) . A Study on the Structure , Function Relation Ship of Lipopeptide Biosurfactant . *Biochim . Biophys Acta.*, 1488:211-218 .
- [7] Jain, D.K.; Thompson , D.L.C.; Lee , H. and Trevors , J.T.(1991) . A Drop Collapsing Test for Screening Surfactant–Producing Microorganisms . *J. Microb. Met .*, 13 : 271-279 .
- [8] Cooper,D.G. and Goldenberg ,B.G . (1987). Surface –Active Agents from Two *Bacillus* species. *Appl.Environ. Microbiol .*,53(2):224-229.
- [9] Plaza,G.A.; Zjawiony,I. and Banat ,I.M. (2006).Use of Different Methods for Detection of Thermophilic Biosurfactant Producing Bacteria from Hydrocarbon Contaminated Soils . *J. Pet. Sci.Eng .*, 50(1): 71-77.
- [10] Patil ,S.D. and Shendre ,L.P.(2018). Comparative Study of Biosurfactant Producing Organisms Isolated from Pesticide and Oil Contaminated Soil . *Int. J. Res. Appl. Sci. Eng. Technol. (IJRASET)* .,6(IV) :2220-2228 .

عزل وتشخيص البكتريا المنتجة لمنشطات السطح الحيوية من الترب الملوثة بالنفط الخام في حقل الرميلة النفطي

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الخلاصة

تشمل الدراسة الحالية عزل وتشخيص نوعين من البكتريا من الترب الملوثة بالنفط الخام في حقل الرميلة النفطي في محافظة البصرة والتي لها القدرة على إنتاج منشطات السطح الحيوية وهذه الانواع هي الكوكوريا كرسطينيا وجنس الرودوكوكاس . أبدى النوع البكتيري الكوكوريا كرسطينيا والذي عزل لأول مرة بالعراق كفاءة عالية في اختبار مؤشر الاستحلاب بنسبة 66% ومنطقة صافية بقطر 8 سم في اختبار أنتشار النفط تلاه جنس الرودوكوكاس بنسبة 50% في اختبار مؤشر الاستحلاب و 7.5 سم في اختبار أنتشار النفط . نستنتج من ذلك أن هناك إمكانية لإستخدام هذه الانواع الفعالة في المعالجة الحيوية للترب الملوثة بالنفط الخام ومشتقاته .

الكلمات المفتاحية : منشطات السطح الحيوية , اختبار إنهيال القطرة , التربة الملوثة , اختبار مؤشر الاستحلاب , اختبار إنتشار النفط .