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Performance of Isolated Kocuria sp. SAR1 in Light Crude Oil Biodegradation

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Abstract

In the current study; Kocuria sp. SAR1 was isolated from 'Tobruk Refinery' oil water s[illegible] coast of Libya. The isolated bacterial strain SAR1 was characterized as an aerobic, Gram +ve, spherical-shaped, oxidase – but catalase +. Phenotypic characters and phylogenetic analysis based on the 16S rRNA gene of the isolate SAR1 showed that it was related to members of the Kocuria genus. The alignment of the 16S rRNA gene sequences of SAR1 with sequences obtained by doing a Blast searching revealed 96% similarity to Kocuria palustris strain TAGA27. Solid waste dates (SWD) and corn steep liquor (CSL) as agro-industrial products were performed to enhance the performance of Kocuria sp. SAR1 in crude oil biodegradation. During bacterial growth, high emulsifying activity to the presence of cells was observed, which is concluding the production of bio surfactant by strain SAR1. The bacterial strain [illegible] crude oil in [illegible] days when cultiv[illegible] (w/v) of CSL and SWD, respectively. Crude oil metabolizing bacterium can secrete surfactants using agro industrial as substrates, which further enhance the hydrocarbon degradation.

Keywords: Isolation; Identification; 16S Rdna; Biodegradation; Crude oil; SWD

Introduction

The ocean and other water bodies have captured the imagination of people for thousands of years. Most of the life forms exist mostly in these water bodies and therefore, specific laws, and regulations have been framed to protect this delicate marine environment. It is also the major source of food chain and is known for diversity of aquatic species [1,2]. Marine oil pollution occurs when any organic or toxic chemical substances enter the sea water. These chemical hazards can lead to severe pollution of the system either for short duration or over long period of time. Primarily it affects the biological process thereby damaging the marine life cycle. Most common causes of marine pollution are due to oil spill occurring at sea. These oil spills deteriorate the marine environment to such an extent that, it destroys the existing ecosystem accordingly affecting the bio diversity and human wellbeing [3-5]. The oil spill results in significant changes in its physical and chemical structure [6,7]. The photolysis of oil can result in the formation of many byproducts such as the aromatic oxygenated compounds, aliphatic, benzoic and naphthanoic acids, alcohols, phenols and aliphatic ketones [8,9].

In Libya, 5 oil terminal facilities and many different operating companies are discharging effluents at risk rate. Oil pollution presents the hazard along parts of the Libyan Marine Coast where Oil Industries are located. This severely affects ecosystem. With the focus on the protection of environment and pollution control in Libya, most of the terminal facilities with the conventional ballast water process are likely to face a serious threat in coming years and need has been felt for a safe, environmental friendly process which eliminates the use of discharging of ballast water into sea. At present, bioremediation use of microorganisms to remove pollutants is often the most suitable method for remediation of especially petroleum hydrocarbons, because it is cost-effective and, it converts the petroleum hydrocarbons into the harmless by-products such as carbon dioxide and water [10,11]. Various microbial populations, including Bacteria, Fungi and Algae can metabolize the hydrocarbons found in crude oil. In literature, bacterium was considered to be the most important group of petroleum degrading organisms because its allow adaptation to various environments, have a rich taxonomic, metabolic, physiological, and it has more rapid metabolic rates. Moreover, bacteria can be genetically manipulated to improve their bioremediation capabilities [12,13]. There are at least 175 genera of bacteria that can metabolize petroleum hydrocarbons, which include- Pseudomonas, Aeromonas, Bacillus, Flavobacterium, Corynebacterium, Micrococcus etc. [14]. Based on crude oil degradation capacity, Pseudomonas aeruginosa is the most active hydrocarbon utilize in crude oil. Previous observations have identified the Pseudomonas genus most efficient among hydrocarbon degrading microorganisms [15-17].

Further use of surfactants has been found to enhance degradation of crude oil [18,19]. Among various surfactants, rhamnolipids are considered to be the most efficient way in degrading hydrocarbons [20-22]. Most studies reported the effectiveness of using consortium bacterium compared with the single strain bacteria. Single strain is still not effective in terms of biodegradability. Both consortium and single strains reported limited performance when used in high crude oil concentration. The isolated Kocuria sp. from contaminated water by crude oil was recently received attention in crude oil biodegradation [23]. However, the success of their production depends on the increase of yield, the development of economic biotechnology processes, and the use of low cost effective renewable agro-industrial substrates for their production. However, the performance of employing SWD and CSL as a low-cost material to enhance single strain bacteria in removing highly contaminated crude oil was not well investigated. The aim of this

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study is to evaluate the e ectiveness of isolated Kocuria sp. on crude oil biodegradation.

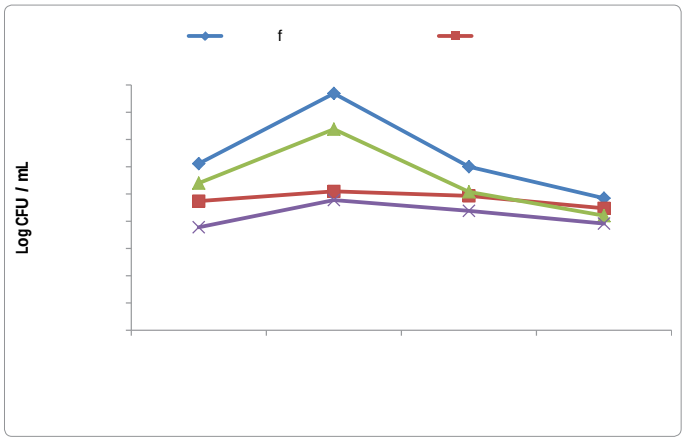
Materials and Methods

Sampling

In the present study, Oil contaminated water samples were collected from Tobruk Re nery (Libya) Figure 1, where Arabian Gulf Oil Co. Company (AGOCO.) It has produced huge amount of crude oil. Samples were collected in an autoclaved vial by using simple sampling technique. Care was taken in handling and sampling to avoid contamination of the samples and returned to the laboratory for bacterial isolation immediately. If necessary, the sample vials were held under refrigeration at 4°C until isolation, which was no later than 48 hours a er sampling.

Isolation of hydrocarbon-degrading bacteria

Collected samples were serially diluted and plated onto a basal medium amended with 0.5% (w/v) crude oil hydrocarbon as the sole carbon source. Flasks were incubated at 30°C on a rotary shaker (150 rpm). 10 mL from each ask was transferred separately to fresh asks containing 90 mL of Enrichment media, and the procedure was repeated for three transfers namely; 7, 14, and 28 days, respectively. Serial dilutions (10^{-7}) of each transfer were inoculated on TGY medium plates to enumerate TCFU and onto BSM-Crude Oil plates to count BDM. Plates were incubated at 30°C, and colonies were enumerated a er 48 h on TGY plates and a er 168 h on BSM-Crude Oil plates. Separate colonies from BSM-Crude Oil plates were picked and puri ed on BSM-Crude Oil plates. Individual colonies were streaked successively onto the same medium (basal medium with 0.5 (w/v) crude oil hydrocarbons for isolation and puri cation [24]. e basal Salt Mineral (BSM) medium according to Piddington CS [25], was prepared by dissolving 2.44 g KH_2PO_4, 5.57 g $NaHPO_4$ 2.0 g NH_4Cl, 0.2 g $MgCl_2 6H_2O$, 0.001 g $FeCl_3 6H_2O$, 0.001 g $CaCl_2 2H_2O$ in one liter sea water and pH was adjusted to 7.0 using 10% NaOH, blended with 0.5% (w/v) Sarir crude oil was used as carbon source and autoclaved for 20 minutes at 120°C. For isolation and enumeration of total viable cells; BSM with crude oil 0.5% (w/v) and TGY were solidi ed by 2% agar and plates were used. Each medium was autoclaved for 20 minutes at 120°C. According to Benson HJ [26] Trypyon glucose yeast extracted medium (TGY) was prepared by dissolving 5.0 g Trypton, 3 g yeast extracts, and 1 g glucose in one liter double distilled water and autoclaved. For the total viable count; each medium pH was adjusted to 7.0 using 10% NaOH.



Local Solid waste date (SWD) was purchased from Benghazi local market, Libya, and its compositions were (as reported by manufacturer, for each 100 ml : Moisture, 13-16%; Protein, 1.0-2.3%; Ash, 1.4-1.8%; total sugar, 75-76% (Glucose: 38%; Fructose: 36%) - Pectin, 0.1-0.2%; Tannin, 0.2-0.3%; pH, 4.5-5.3; TDS, 74-75%. Corn steep liquor (CSL) used for this study was provided from El-Nasr Pharmaceutical Company; carbon: nitrogen: phosphorus content of CSL was 6.8: 1.06: 2.14 (% w w^{-1}), respectively.

Identi cation and characterization of bacteria

Biochemical characterization: To identify and characterize the bacteria isolates, the designed Strain SAR1 colonies were identi ed by a combination of information from primary and secondary identi cation. Morphological, physiological and biochemical characteristics of pure isolated were examined according to the Bergey's Manual of Determinative Bacteriology. Primary identi cation was done based on colony and cell morphology and Gram staining [27]. Representative colonies of strain SAR1 appeared on plates were checked for purity through the microscopy and pure isolates were streaked on slants of BSM-Crude Oil medium on which they developed during isolation and stored at -40°C for further investigation. For secondary identi cation, Bacterial isolates was characterized by 16S rRNA gene sequencing [28]. A er identi cation the isolate was deposited in the culture collection for long-term preservation. DNA isolation and ampli cation were done in Sigma, as follows;

a. DNA Isolation: e most e cient biodegrading bacterial isolate was incubated separately in TGY for 48 h at 30°C on a rotatory shaker (150 rpm). e genomic DNA was extracted using Gene JET Genomic DNA Puri cation Kit K0729 (Fermentas, USA).

b. PCR Ampli cation of 16S rDNA Regions: Two sets of primers were used to amplify regions speci c for almost all eubacteria 16S rDNA sequences. e 16SrDNA gene was ampli ed by polymerase chain reaction (PCR) using the following primers [28]. e universal primers Fd1 and Rd1 (Fd1,5-TGCCTGGTAGTGGGGGATAA and Rd1,3'- CCAGGTAAGGTTCTCGCGTT-3').

e reaction was prepared with 0.5 µL of Dream-Taq 5 U/µL (Fermentas, USA), 5 µL of Dream-Taq Bu er 10x, 5 µL of target DNA, 1 µL of dNTP each 20 mM, 1 µL of each appropriate primer 10 pmol/µL and 36.5 µL dH_2O were added. e nal reaction volume was 50 µL.

e PCR condition was as follows:

Step 1: Pre-Denaturation: 94°C 5 min.

Step 2: 35 cycles of:

- Denaturation 94°C 40 sec.
- Annealing 48°C 40 sec.
- Extension 72°C 3 min.
- Final Extension 72°C 10 min

Step 3: Hold at: 4°C Inde nitely

A er completion of PCR program, the visualization of PCR products was carried out using, 5 L of the suspension electrophoresed on 1% agarose gels in 1X Tris-Acetate EDTA (TAE) bu er, which were then stained with ethidium bromide and examined under UV light. Bands were excised, and DNA was puri ed from gel slices using QIAquick Gel Extraction Kit, Cat. No. 28704 (Qiagen, USA). e puri ed PCR products were sequenced with the same primer that has

been used in ampli cation of the target sequence. Sequencing was done by an ABI 3730 XL automatic DNA sequencer at Sigma, Giza, Egypt. e 16S rDNA sequences (Query sequence) were initially analyzed at NCBI server (http://www.ncbi.nlm.nih.org) using BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and corresponding sequences from database were downloaded. Evolutionary history was inferred using the Neighbor-joining method [29]. e tree was drawn to the scale, with branch lengths in the same units as those of the evolutionary distance used to infer the phylogenetic tree.

Crude oil-biodegradability assay: Biodegradation assays were also performed in liquid culture to examine the ability of isolated microorganisms to utilize crude oil as a sole energy source according to Ismail et al. [30]. is assay was done as follows: Cells were incubated at 30°C in TGY for 24 h in a shaking incubator (150 rpm). Next, cells were pelleted by centrifugation at 5000 rpm for 15 min and then washed three times with BSM. Washed cells were inoculated into BSM that contained crude oil as a sole energy source, to test the ability of isolates to degrade crude oil (Crude Oil-biodegradability assay). e inoculum was adjusted so that the beginning absorbance was (A600 0.35uligd a37 Tw -30xoP7 pn152 Tw T* (was axTw 0 -1-1 8411med was

where D_1 is the concentration of hexane extractable material in the sample and D_2 is the concentration of hexane extractable material in the duplicate sample.

Results and Discussion

Isolation and identi cation of the bacteria

Produced water sample were collected from the oil contaminated site showed density in the range of 1.3×10^{9} CFU/ml to 1.7×10^{10} CFU/ml. Subsequently, the nal growth rate of 2.1×10^{10} CFU/ml to 1.1×10^{11} CFU/mL (CFU: colony forming unit) were measured for crude oil, enhanced with CSL and SWD respectively. us concluded the presence of visual emulsi cation was found to be growth associated in strain SAR1, where a parallel relationship exists between growth substrate utilization and emulsi cation activity. However, Xu et al. [33] reported that there was no decrease in the CFU of strain SY23 during the degradation even at higher concentration of crude oil. ese results indicated that the bacterial strain SAR1 supported growth even at higher concentration. An enrichment culture initiated in basal medium containing 0.5% (w/v) crude oil as the carbon and energy source became turbid and the dark layer of crude oil became clear, indicating the degradation of this substrate. e obvious increase in the count of BDM and TCFU a er Enrichment for one week in Enrichment medium, illustrated in Figure 1, might be due to the adaptation of the indigenous microbial populations. e viable counts of BDM either before or a er Enrichment were less than TCFU indicating that; not all the indigenous microorganisms have the enzymatic system capable to degrade crude oil hydrocarbon and only microorganisms that have the required enzymatic system to metabolize crude oil would grow on the BSM/ containing crude oil. Enrichment culture showed the presence of aerobic bacteria with diverse cellular morphologies and then was used in the isolation of pure bacterial strains in solid media. One colony was picked, and a pure culture designed SAR1 was selected (Figure 2).

is culturing technique based upon the principle that when material-containing microorganism is cultured each viable microorganism will develop into a colony [34]. It is known that Gram (+) ve isolate designated as SAR3 belonged to the genus *Kocuria*. e Gram positive bacterium had spherical shape; opaque, moderate with entire edges occurred in groups. It was aerobic, oxidase -negative, but catalase- positive. Enrichment culture showed the presence of aerobic bacteria with diverse cellular morphologies, which was isolated in solid media to obtain pure bacterial strains as shown in Figure 2.

e strain was identi ed as Kocuria sp. strain SAR1 and water-soluble pigments that gave it a characteristic yellow-orange. e selection of the strain was based on its high capacity to degrade crude oil, in solid and liquid media. e strain SAR1 was used for further characterization. is strain was rst identi ed using classical biochemical morphological characteristics as shown in Figure 2. e

low cost substrates, such as molasses and corn steep liquor, for use in environmental applications. e addition of both the biosurfactant and/ or bacterial cells of P. cepacia favored the biodegradation of hydrophobic organic compounds. It is evident to the results that the biosurfactant alone, and its producer species are both capable of stimulating biodegradation to a large extent. us, Silva EJ et al. and Nikolopoulou et al. [35,36] have reported that identifying the key organisms that play an important role in di erent bioremediation treatments for understanding, evaluating and further decide upon the best in situ bioremediation strategy. To reduce medium costs of date's molasses, and corn steep liquor (CSL), a by-product of wet milling corn for starch, are extensively used in bioremediation as a component of microorganism culture medium. It provides a rich but economical source of nutrients, rich sugar, amino acids, organic acids, vitamins, and minerals [37,38].

Even though the process is site speci c to Libya, however, aspects of isolation process, DNA identi cation and biodegradation experimental methods can also be applied to other similar hydrocarbons properties elsewhere.

Conclusion

Bacteria strain SAR1 was isolated and characterized belonging to the genus Kocuria according to its 16S rDNA, as well as biochemical characteristics. DNA–DNA relatedness indicates that strain SAR1 is a member of the same genomic species. It is Gram-positive, large opaque, shiny colonies with serrated edges, occurred in pairs and sometimes in chains or in a group, aerobic, oxidase-negative, catalase positive. Kocuria sp. strain SAR1 can utilize crude oil hydrocarbons as the sole carbon source. Strain SAR1 has a high ability to degrade crude oil in a pH 7, temperature 30 C°. e degradation e ciencies and the result showed that the isolate could remove 68%, 70% in 28 days when cultivated with 0.2% (w/v) of solid waste dates and corn steep liquor, respectively. e use of pretreated solid waste dates which forms a by-product of the agro- industry as the state of Libya has an easy access t enhance the bioremediation of oil spills, may form a useful method to be applied.

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References

1. Soma K, Ramos J, Bergh, ST, van Oostenbrugge H, van Duijn AP et al. (2013) The “mapping out” approach: effectiveness of marine spatial management

15. Lal B, Khanna S (1996) Degradation of crude oil by Acinetobacter calcoaceticus and Alcaligenes odorans. J Appl Bacteriol 81: 355-362.

16. Saadoun I (2002) Isolation and characterisation of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel fuel. J Basic Microbiol 42: 420-428.

17. Banat JM, Makkar RS, Cameotra SS (2000) Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 53: 495-508.

18. Urum K, Pekdemir T, Gopur M (2003) Optimum conditions for washing of crude oil-contaminated soil with biosurfactant solutions. Process Safety and Environm Protect 81: 203-209.

19. Yateem A, Balba M T, Al-Shayji Y, Al-Awadhi N (2002) Isolation and characterization of biosurfactant-producing bacteria from oil-contaminated soil. Soil and Sediment Contamination 11: 41- 55.

20. Itoh S, Suzuki T (1972) Effect of rhamnolipids on growth of Pseudomonas DHUXJLQRVD PXWDQW GH¿FLHQW LQ Q SDUDI¿Q XWLOL]LQJ DELOLW\ $JULF %LRO &KHP 2233-2235.

21. Andrä J, Rademann J, Hoza6D0Of1Riol 4ag458 Tm (C2zs 7 0 3ization)-0.8dEndotoxin-lik.156 Tw 59.083 -4m (7d [(petroleuropertuelof)]TJ 0 alipids)-1.7exotoxinon