VOL.66 NO.01 JANUARY-JUNE 2024 • PRINT ISSN 0025-3146 • ONLINE ISSN 2321-7898

JMBAI

JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF INDIA

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Marine Biological Association of India
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Available online at: www.mbai.org.in doi:10.6024/jmbai.2024.66.1.2455-14

Biosurfactant producing bacteria, *Bacillus halosaccharovorans,* from a marine ecosystem

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Received: 30 Oct 2023 Revised: 01 Mar 2024
Accepted: 05 Mar 2024 Published: 09 May 2024

Original Article

Abstract

Biosurfactant-producing microorganisms show potential for bioremediation of oil from soil and water surfaces. These hydrocarbonoclastic microorganisms release biosurfactants, which play a significant role in the bioremediation of oil. The present study focuses on various isolation and screening strategies for biosurfactant-producing organisms from oil-polluted sites. A total of eight isolates showing morphological variations were isolated and screened for biosurfactant production using various tests like Hemolysis assay, Drop collapse assay, Oil displacement assay, Microtiter plate assay, Emulsification index assay, and Phenol: Sulphuric acid assay. The potent biosurfactant producer was identified as *Bacillus halosaccharovorans* with 16s rRNA sequencing. FTIR analysis was performed on the extracted biosurfactant. To our knowledge, this paper is the first to demonstrate that *Bacillus halosaccharovorans* is a biosurfactant producer.

Keywords: *Biosurfactant, emulsification index, Bacillus halosaccharovorans, FTIR, marine ecosystem*

Introduction

Oil spills exhibit a great threat to marine flora and fauna due to their inherent chemical composition as well as their ability to obstruct sunlight penetrating the oceans. It can coat the wings of birds and render them unable to fly. Even the small concentrations of Poly aromatic hydrocarbons (PAHs) can have sub-lethal to lethal damage to marine biology. It can also lead to oxygen deficiency within the affected marine water (Amir-Heidari *et al.,* 2019). In humans, it can lead to imbalances in the endocrine system, and respiratory malfunction and may lead to kidney, skin, lung, liver, or bladder cancer (Deosthali *et al.*, 2021). Barron *et al.,* 2020 have compiled the consequences of the most significant worldwide oil spills in the past three decades, namely the Exxon Valdez oil spill, the Hebei Spirit oil spill, and the Deepwater Horizon oil spill. The number of affected biological species speaks of the grave danger an oil spill poses to the marine environment (Barron *et al.,* 2020).

Strategies like the use of booms, adsorbents, skimmers, chemical dispersants, and in-situ burning are being employed to tackle the oil spills. However, each of these strategies comes with its disadvantages and thus the last resort becomes bioremediation (Dave and Ghaly, 2011; Al-Majed *et al.,* 2012; Akpor *et al.,* 2014). Bioremediation by the means of microorganisms enables the eco-friendly pathway for the elimination of pollutants. Hydrocarbonoclastic bacteria (a heterogeneous group of prokaryotes, which can degrade and utilize hydrocarbon compounds as a source of carbon and energy) produce amphiphilic molecules called biosurfactants. These biosurfactants facilitate the bioavailability of oil to bacteria by reducing the interfacial tension between oil and water surfaces (Sajadi *et al.,* 2022).

Biosurfactants have a wide array of applications, other than oil spill bioremediation. Its antioxidant, antimicrobial, and wound-healing abilities make it a suitable candidate for the pharmaceutical industry. The ability to increase the bioavailability of trace metals and growth-promoting properties has paved its path in the agricultural industry. The property to reduce surface tension has enabled biosurfactants to compete with commercially available chemical surfactants, thus having potential applications in the detergent and cleaning industry (Bouassida *et al.,* 2017).

Material and methods

Sample collection and Storage

Samples were collected at multiple sites, resulting in a total of five samples contaminated with oil in both water and soil.

These samples were collected in sterile containers and were transported to the lab in an ice bath. The samples were stored at 4 °C till further processing.

Media: Media used for enrichment and isolation of biosurfactant-producing bacteria is Bushnell and Hass broth/ agar (HiMedia). It is a preferable medium for the growth of marine microorganisms due to its salt composition and it gives freedom for the selection of carbon source. It was modified by adding 3% NaCl for selective isolation of halotolerant bacteria. Blood required for blood agar plates was purchased from Central Hospital Blood Bank, Ulhasnagar. All the chemicals required were purchased from Loba Chemie Pvt. Ltd. and were of analytical grade.

Enrichment and Isolation: The enrichment of samples was carried out in three stages. In the first stage, 1g of soil, 1 ml of liquid sample, and 1 ml of suspension (for sample 4) were inoculated in 100 ml sterile MBHB broths containing 0.5% of phenol as carbon source and were incubated at room temperature on an orbital shaker for seven days. In the second stage, 1 ml of inoculum from the 1st stage flasks were inoculated in sterile MBHB containing 1% phenol as a carbon source. In the last stage, the 1 ml of inoculum from the second stage was inoculated in sterile MBHB with 1% spent ship oil (SSO) as a carbon source and was incubated at room temperature for seven days on an orbital shaker. For isolation, sterile MBHA plates with 1% SSO were used. A loopful of broth was streaked on the plates and they were incubated at room temperature for 72 hours (Ezebuiro *et al.,* 2019).

Retrieval of Cell-Free Supernatant (CFS): The isolates obtained in pure form were inoculated in sterile MBHB with 1% SSO and were allowed to grow at room temperature for 72 hours in an orbital shaker. The cell mass was separated by centrifuging at growth media at 6000 rpm at 4°C for 15 mins. The resulting CFS was used to screen the biosurfactant production (Abbasi *et al.,* 2012).

Screening of Biosurfactant producing microorganisms: Hemolysis assay was done through a loopful of pure culture from MBHB was streaked onto blood agar plates. After incubating at room temperature for 24 hours, plates were examined for hemolysis patterns. (Ogunshe and Falode, 2021). For the phenol sulphuric assay, 1 ml of 5% phenol was mixed with 1 ml of CFS and vortexed thoroughly. This was followed by the drop-wise addition of conc. H_2SO_4 along the side of the tube. The formation of an orange colour indicated a positive response (Suresh *et al.,* 2021). For the microtiter plate assay, 100 µL of CFS was added to wells of a 96-well microtiter plate. A graph paper was kept under the plate and was observed for distortion of grid lines. Distilled water in a hydrophobic well exhibits a flat surface. If biosurfactant is present then the image of the grid will be distorted due to the concave surface (Saruni *et al.,* 2019). For the drop collapse assay, an oil film was applied to a glass slide to create a hydrophobic surface. A drop of CFS was placed on the slide and it was checked whether it remained in bead shape or collapsed. Loss of shape or flattening of drop indicates a positive response (Sohail and Jamil, 2019). For the oil displacement assay, a Petri plate was prepared for this assay by filling it with 20 ml of distilled water. It was overlaid with 15 µL of oil. 10 µL of CFS was placed on the oil surface. The formation of a clear zone resulting from the spread of oil by reduced surface tension indicated a positive test for biosurfactant (Kurniati *et al.,* 2019). For the Emulsification Index Assay, 2.5 ml of CFS was added to a tube having an equal volume of SSO and was vortexed vigorously for 3 minutes. It was left undisturbed for 24 hours at room temperature. The emulsification index was determined as a percentage by dividing the height of the emulsified layer by the height of the liquid column and multiplying it by a hundred (Zargar *et al.,* 2022).

Identification of potent biosurfactant producer: For the biochemical test, gram nature of potent isolate was identified and accordingly, standard biochemical tests were carried out. We utilized Bergey's manual of systematic bacteriology to compile and identify the genus of the organism based on the obtained results (Singh *et al.,* 2016). For 16s rRNA sequencing, the potent isolate selected was identified by 16s rRNA sequencing and a phylogenetic tree was generated (Deosthali and Jain, 2022).

Extraction of crude biosurfactant: The potent isolate was inoculated in sterile MBHB with 1% SSO and was incubated on an orbital shaker at ambient temperature for a week. Post incubation, the cell-free supernatant (CFS) was acquired through centrifugation of the complete culture medium at 6000 rpm and 4°C for 15 minutes. Then the CFS was acidified using 6N HCL till a pH of 2.0 was achieved. It was kept at 4°C for 24 hours to facilitate maximum precipitation. The pellet was obtained by centrifuging the content at 4°C, 12500 rpm for 20 mins. The pellet was resuspended in distilled water and the pH was raised to 7.0 with the help of 1N NaOH. We performed an extraction on this suspension using methanol. The methanol portion was transferred to a beaker and left to evaporate at

Table 1. Geographic coordinates of the sampling sites

No.	Sampling site	Geographical coordinates	Sample type
1	HP Petrol Pump	19° 10' 15.672" N 73° 4' 54.696" E	Soil
2	Indian Oil Petrol Pump	19° 8' 21.732" N 73° 3' 0.684" E	Soil
3	Reti Bunder, Dombivli	19° 13' 45.0876" N 73° 4' 4.9944" E	Water
4	Below New Kon Bridge, Kalyan	19° 14' 45.0384" N 73° 7' 3.7164" E	Water + Soil
5	Mumbai High	19° 25' 0.012" N 71° 19' 59.988" E	Water

37°C. A second round of methanol extraction was carried out, resulting in the isolation of crude biosurfactants (Das *et al.,* 2008). For obtaining the FTIR of crude biosurfactant, 2 mg of crude biosurfactant was mixed with 20 mg KBr pellet. The FTIR was performed from a range of 400 to 4000/cm using IR Prestige-21 (Shimadzu) (Devaraj *et al.,* 2019).

Results

From all five different samples, eight morphologically different isolates were obtained on MBHA with 1% SSO. These isolates were subjected to screening methods as described above. For all the tests a positive control as well as negative control was maintained with Triton X-100 (1 mg/mL) and Distilled water respectively. CD08A, CD011A, and CD11C showed a beta hemolysis pattern whereas other isolates showed an alpha hemolysis pattern. All the isolates showed a positive reaction for Phenol: Sulphuric acid test by forming an orange-coloured product. The Microtiter plate assay relies on biosurfactants' capacity to decrease surface tension, causing deformation of the grid positioned beneath it. All the isolates showed a distortion effect when compared with distilled water. The CFS containing biosurfactant reduces the surface tension between oil and water interface enabling the drop to spread more than that of negative control. In drop collapse assay, the CFS of CD11C showed the largest diameter of 5 mm which was 1 mm larger than positive control. The rest of the isolates showed a diameter within a range of 2-3 mm with negative control showing a drop diameter of 1 mm. The formation of the clear zone on the oil surface in the oil displacement assay is scored as shown in Table 2. CD11C showed the highest score for oil displacement followed by CD08A and CD11A. Triton X-100 showed an emulsification index of 38.46%. CD12A and CD09A showed an emulsification index of less than 40% whereas the rest of the isolates showed an emulsification index of more than 40% with CD11C having an E_{24} of 44.11%.

Table 2. Results for DCT, ODT, and E_{24} test

Samples	Drop Collapse Test	Oil Displacement Test	Emulsification Index Assay
CD08A	3 mm	+ + + +	43.75 %
CD08B	2 mm	+	43.75 %
CD09A	2 mm	+	39.39 %
CD10A	3 mm	+	43.75 %
CD11A	3 mm	+ + +	42.30 %
CD11B	2 mm	+	41.93 %
CD11C	5 mm	+ + + + +	44.11 %
CD12A	2.5 mm	+	35.71 %
NEG CNTRL	1 mm	-	0.0 %
POS CNTRL	4 mm	+ + + + +	38.46 %

We identified CD11C as a promising candidate and conducted a series of biochemical tests on it. The results of the biochemical tests are mentioned in Table 3. The results concluded that the organism belonged to the *Bacillus* genus. The results of 16s rRNA sequencing were compared with the present sequence database using NCBI BLAST and a phylogenetic tree was also generated (Fig 1). The isolate CD11C showed 98.17% similarity with *B. halosaccharovorans*. The 16s rRNA sequence was deposited in NCBI GenBank under the accession number OL989233.

The partially purified biosurfactant (Fig. 2) was subjected to FTIR analysis (Fig. 3). A broad peak can be observed in the FTIR graph of CD11C biosurfactant within the range 3000–3500 cm^{-1}, which can be the result of alcohol (-OH) group. Two sharp peaks within the range 2850 – 3000/cm, indicate the presence of sp3 C-H stretching. Two sharp peaks at 1350/cm and 1362/cm indicate the presence of the nitro group in the compound. A sharp peak at 1126/cm indicates the presence of the alkoxy (C-O) group. A group of peaks between 980 and 1300 resemble the chemical structures of rhamnose rings (Bertuso *et al.*, 2022).

Discussion

Studies reflect that around two million tons of oil are introduced into the aquatic environment per year due to activities carried out in the sea (Zahed *et al.,* 2022). The current strategies employed for the removal of oil use booms, skimmers, and chemical dispersants which are ok to be used in deep water.

Table 3. Results from the Biochemical Test

Test	Result
Gram Stain	Gram Positive
Motility	+
Indole	-
MR	-
VP	+
Citrate	-
Nitrate Reduction	-
Catalase	+
Oxidase	+
Urease	-
LDC	+/-
Glucose	+
Fructose	+
Sucrose	+
Lactose	+
Xylose	+
Mannitol	+

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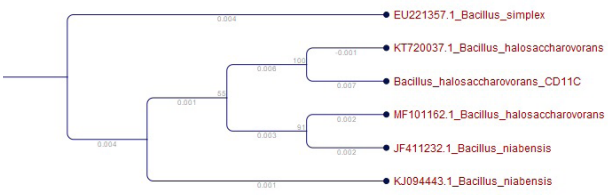

Fig. 1. Phylogenetic tree for CD11C

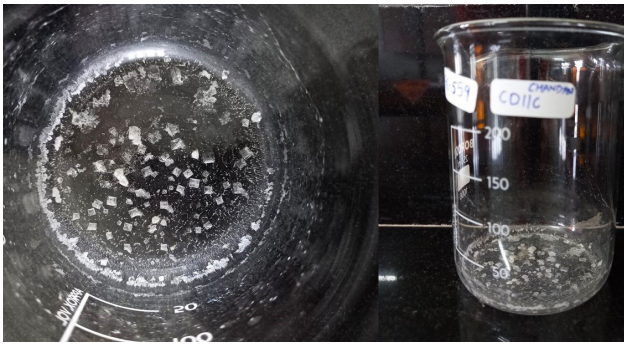

Fig. 2. Partially purified biosurfactant

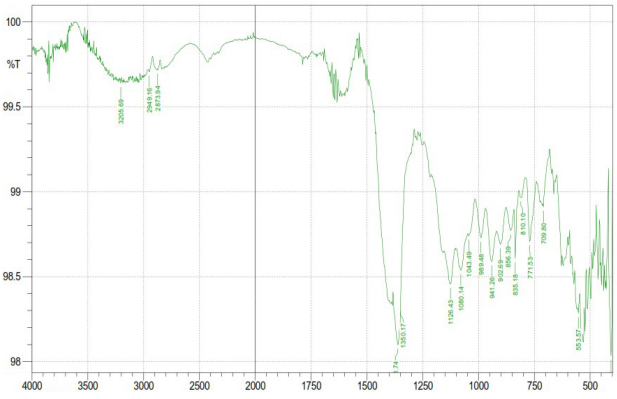

Fig. 3. FTIR analysis of CD11C Biosurfactant

The use of chemical dispersants is not recommended near the shore water as it may damage the local flora and fauna living in shallow waters. Chemical fertilizers were used near the shoreline during two of the major oil spills *i.e.* in the Gulf of Mexico-Deepwater Horizon oil spill and in the Gulf of Alaska-Exxon Valdez oil spill. However, these chemical fertilizers just disperse the oil and make it available to the local oil-degrading bacterial community and do not enhance the rate of its degradation (Atlas and Hazen, 2011). 3.1.6 of Policy and Guidelines For the use of Oil Spill Dispersants (OSD) In Indian Waters, published in NOS-DCP (2009) prepared by the Indian Coast Guard dictates that no oil dispersant shall be used in shallow water, protected bays, and inlets. However, no direct application of oil-degrading bacteria has been reported yet.

The potential threats of oil spills on marine biodiversity urge the scientific community to come up with faster and more eco-friendly bioremediation solutions. The objective of this research was to recover bacteria capable of producing biosurfactants and degrading oil from sites contaminated with petroleum. We aimed to assess their potential application in the field of bioremediation technology. Oil-contaminated sites provide a selective strategy to isolate hydrocarbonoclastic microorganisms which have the required genetic material to process oil as their carbon source (Adeleye *et al.,* 2022). Harikrishnan *et al.* (2021) isolated six morphologically different isolates on oil agar plates from oil-contaminated soil samples in Karaikal ONGC, Puducherry, India (Harikrishnan *et al.,* 2021). Jagtap *et al.* (2021) were able to isolate obligate hydrocarbonoclastic organisms from oil-contaminated water sites in the Arabian Sea (Jagtap *et al.*, 2021). Devi and Jha successfully isolated 36 oil-degrading bacteria from refinery sludge samples of an oil refinery (Devi and Jha, 2020). Thus, it can be implemented that even though biosurfactant-producing organisms are ubiquitous, the oil-contaminated sites provide a reliable source to isolate potent hydrocarbonoclastic, biosurfactant-producing bacteria. The biosurfactant-producing *Bacillus* species have been reported before from various sources including soil, rhizosphere, and marine ecosystems. Bartal *et al.* (2023), isolated different *Bacillus* species capable of producing surfactin-like biosurfactants from the rhizosphere. This includes, *B. cereus, B. atrophaeus*, *B. subtilis*, *B. pumilis* and *B. velezensis*. *Bacillus licheniformis* Ali5 was reported to remove around 70-79% of motor oil from sand with the help of biosurfactant produced by it. It resulted in an additional 32% oil recovery when the biosurfactant was applied to a sand-packed column (Ali *et al.*, 2019). *Bacillus cereus* was isolated from marine seawater and showed 96% degradation of motor oil within 27 days and the biosurfactant generated exhibited stability across a broad pH spectrum, temperature, and salinity (Durval *et al.*, 2019). The antifouling property of biosurfactant extracted from *B. niabensis* was studied against phytoplanktonic cells and biofilm-forming bacteria like *Bacillus subtilis*, *Micrococcus* sp., *Sagittula stellata* and *P. stutzeri* (Alemán-Vega *et al.*, 2020; Sánchez-Lozano *et al.*, 2023). However, the study on the biosurfactant-producing capabilities of organisms closely related to CD11C which are *B. simplex* and *B. niabensis* are not well studied for their bioremediation potential and to the best of our knowledge this is the first paper shedding light on biosurfactant production by *B. halosaccharovorans*.

Conclusion

The successful isolation of hydrocarbonoclastic bacteria confirms the hypothesis that oil-contaminated soil and water are potential sites for isolating biosurfactant-producing and oil-degrading bacteria. The bacteria isolated were screened

for their biosurfactant-producing ability and the most promising isolate was identified as *B. halosaccharovorans* using 16s rRNA sequencing. The revelation of rhamnose rings in the FTIR graph of a partially purified biosurfactant isolated from CD11C indicates that the biosurfactant produced might be a rhamnolipid. To the best of our knowledge, no previous studies have reported the biosurfactant production by *B. halosaccharovorans*. However, the application of biosurfactants as degreasing agents on different surfaces and the use of oil-degrading bacteria on the field via spray application needs to be investigated further. Also, a study needs to be conducted to investigate and enhance the bioremediation effectiveness.

References

Abbasi, H., M. M. Hamedi, T. B. Lotfabad, H. S. Zahiri, H. Sharafi, F. Masoomi, A. A. Moosavi-Movahedi, A. Ortiz, M. Amanlou and K. A. Noghabi. 2012. Biosurfactant-producing bacterium, *Pseudomonas aeruginosa* MA01 isolated from spoiled apples: physicochemical and structural characteristics of isolated biosurfactant. *J. Biosci. Bioeng.*, 113 (2): 211-219.

Adeleye, A. O., M. B. Yerima, M. E. Nkereuwem, G. O. Onasanya, V. O. Onokebhagbe, G. B. Bate and M. Raji. 2022. Biochemical and PCR-based identification of Hydrocarbonoclastic Bacteria isolated from spent engine oil polluted soil. SLU, *J. Sci. Technol.*, 3 (1&2): 23-34.

Akpor, O. B., U. F. Okolomike, D. T. Olaolu and B. I. Aderiye. 2014. Remediation of polluted wastewater effluents: hydrocarbon removal. *Trend. Appl. Sci. Res.*, 9 (4): 160-173.

Alemán-Vega, M., I. Sánchez-Lozano, C. J. Hernández-Guerrero, C. Hellio and E. T. Quintana. 2020. Exploring antifouling activity of biosurfactants producing marine bacteria isolated from Gulf of California. *Int. J. Mol. Sci.*, 21(17): 6068.

Ali, N., F. Wang, B. Xu, B. Safdar, A. Ullah, M. Naveed, C. Wang and M. T. Rashid. 2019. Production and application of biosurfactant produced by *Bacillus licheniformis* Ali5 in enhanced oil recovery and motor oil removal from contaminated sand. *Molecules*, 24 (24): 4448.

Al-Majed, A. A., A. R. Adebayo and M. E. Hossain. 2012. A sustainable approach to controlling oil spills. *J. Environ. Manage.*, 113: 213-227.

Amir-Heidari, P., L. Arneborg, J. F. Lindgren, A. Lindhe, L. Rosén, M. Raie, L. Axell and I. M. Hassellöv. 2019. A state-of-the-art model for spatial and stochastic oil spill risk assessment: A case study of oil spill from a shipwreck. *Environ. Int.*, 126: 309-320.

Atlas, R. M. and T. C. Hazen. 2011. Oil biodegradation and bioremediation: a tale of the two worst spills in US history. p. 6709-6715.

Barron, M. G., D. N. Vivian, R. A. Heintz and U. H. Yim. 2020. Long-Term Ecological Impacts from Oil Spills: Comparison of *Exxon Valdez*, *Hebei Spirit*, and Deepwater Horizon. *Environ. Sci. Technol.*, 54 (11): 6456-6467.

Bartal, A., T. Huynh, A. Kecskeméti, M. Vörös, O. Kedves, H. Allaga, M. Varga, L. Kredics, C. Vágvölgyi and A. Szekeres. 2023. Identifications of Surfactin-Type Biosurfactants Produced by *Bacillus* Species Isolated from Rhizosphere of Vegetables. *Molecules*, 28 (3): 1172.

Bertuso, P. D. C., C. A. Marangon and M. Nitschke. 2022. Susceptibility of Vegetative Cells and Endospores of Bacillus cereus to Rhamnolipid Biosurfactants and Their Potential Application in Dairy. *Microorganisms*, 10 (9): 1860.

Bouassida, M., N. Fourati, I. Ghazala, S. Ellouze-Chaabouni and D. Ghribi. 2017. Potential application of *Bacillus subtilis* SPB1 biosurfactants in laundry detergent formulations: Compatibility study with detergent ingredients and washing performance. *Eng. Life Sci.*, 18 (1): 70-77.

Das, P., S. Mukherjee and R. Sen. 2008. Antimicrobial potential of a lipopeptide biosurfactant derived from a marine *Bacillus circulans*. *J. Appl. Microbiol.*, 104 (6): 1675-1684.

Dave, D. A. E. G. and A. E. Ghaly. 2011. Remediation technologies for marine oil spills: A critical review and comparative analysis. *Am. J. Env. Sci.*, 7 (5): 423.

Deosthali, C. and A. Jain. 2022. Isolation and screening of microorganisms producing biosurfactants. *J. Mar. Biol. Ass. India*, 64 (2): 94-99.

Deosthali, C., R. Sharma and N. Patil. 2021. Isolation, assessment and identification of potent biosurfactant producing microorganisms from oil contaminated sites. *Int. J. Sci. Res.* 10 (10): 1164-1170.

Devaraj, S., P. C. Sabapathy, L. Nehru and K. Preethi. 2019. Bioprocess optimization and production of biosurfactant from an unexplored substrate: *Parthenium hysterophorus*. Biodegradation, 30: 325-334.

Devi, S. P. and D. K. Jha. 2020. Screening of Bacteria Isolated from Refinery Sludge of Assam for Hydrocarbonoclastic Activities. *J. Pure. Appl. Microbiol.*, 14 (2): 1453-1465.

Durval, I. J. B., A. H. M. Resende, M. A. Figueiredo, J. M. Luna, R. D. Rufino and L. A. Sarubbo. 2019. Studies on biosurfactants produced using *Bacillus cereus* isolated from seawater with biotechnological potential for marine oil- spill bioremediation. *J. Surfact. Deterg.*, 22 (2): 349-363.

Ezebuiro, V., I. J. Otaraku, B. Oruwari and G. C. Okpokwasili. 2019. Effects of nitrogen and carbon sources on biosurfactant production by hydrocarbon-utilizing *Stenotrophomonas* sp. *Microbiol. Res. J. Int.*, 29: 1-10.

Harikrishnan, S., S. Jayalakshmi, M. S. Alsalhi, A. Kartick, S. Devanesan and A. Rajasekar. 2021. Characterization of biosurfactant from pseudomonas stutzeri sj3 for remediation of crude oil-contaminated soil. 02 June 2021, PREPRINT (Version 1) available at Research Square, p. 1-14.

Jagtap, C. B., R. M. Ram, O. K. Tiwari, S. Titus and T. Lodha. 2021. Genome sequence of an obligate hydrocarbonoclastic bacterium Alcanivorax marinus NMRL4 isolated from oil polluted seawater of the Arabian Sea. *Mar. Genomics*, 60: 100875.

Kurniati, T. H., S. Rahayu, D. Sukmawati and W. Maharani. 2019. Screening of biosurfactant producing bacteria from hydrocarbon contaminated soil. *J. Phys. Conference Series,* 1402 (5): 055026.

Ogunshe, A. A. O. and O. A. Falode. 2021. The Intriguing Extrapolations of Haemolysis Assay as Screening Criterion for Selecting Biosurfactant-Producing Microorganisms in Petroleum Industries Process-Conditions. *J. Pet. Environ. Biotechnol.*, 8: 431.

Sajadi, B. M., M. A. Raeisi Estabragh, M. Ohadi, I. M. Banat and G. Dehghannoudeh. 2022. Biosurfactants aided bioremediation mechanisms: A mini-review. *Soil and Sediment Contamination: An International Journal*, 31 (7): 801-817.

Sánchez-Lozano, I., L. C. Muñoz-Cruz, C. Hellio, C. J. Band-Schmidt, Y. Cruz-Narváez, E. Becerra-Martínez and C. J. Hernández-Guerrero. 2023. Metabolomic Insights of Biosurfactant Activity from *Bacillus niabensis* against Planktonic Cells and Biofilm of Pseudomonas stutzeri Involved in Marine Biofouling. *Int. J. Mol. Sci.*, 24 (4):4249.

Saruni, N. H., S. Abdul Razak, S. Habib, S. A. Ahmad, S. A. Alias, W. L. Wan Johari, J. Smykla and N. A. Yasid. 2019. Comparative screening methods for the detection of biosurfactant-producing capability of Antarctic hydrocarbon-degrading *Pseudomonas* sp. *J. Environ. Microbiol. Toxicol.*, 7 (1): 44-47.

Shete, P. and C. Deosthali. 2022. Biosurfactants: Applications and Methodologies. In Advances in Microbiology, Bhumi Publishing. ISBN: 978-93-91768-90-4, 3: 172-186.

Singh, V., S. Haque, H. Singh, J. Verma, K. Vibha, R. Singh, A. Jawed and C. K. Tripathi. 2016. Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. *Front. Microbiol.*, 7: 1921.

Sohail, R. and N. Jamil. 2019. Isolation of biosurfactant producing bacteria from Potwar oil fields: Effect of non-fossil fuel based carbon sources. *Green Processing and Synthesis,* 9 (1): 77-86.

Suresh, A., D. Nagda and J. Abraham. 2021. Screening of biosurfactant producing microorganisms from the soil. Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, *Food Industry*, 22 (4): 411-426.

Zahed, M. A., M. A. Matinvafa, A. Azari and L. Mohajeri. 2022. Biosurfactant, a green and effective solution for bioremediation of petroleum hydrocarbons in the aquatic environment. *Discov. Water,* 2 (1): 5.

Zargar, A. N., S. Mishra, M. Kumar and P. Srivastava. 2022. Isolation and chemical characterization of the biosurfactant produced by *Gordonia* sp. IITR100. *PloS ONE*, 17 (4): e0264202.

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