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Isolation of Marine Bacteria and Evaluation of Crude Oil Degradation Potential

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Abstract

Human activities, including offshore oil exploration and road pollution, have significantly impacted marine ecosystems, with oil spills causing both acute and chronic ecological damage, while indigenous hydrocarbon-degrading bacteria play a key role in mitigating these effects. This study evaluates the crude oil degradation potential of two marine bacterial isolates. Isolate 1 demonstrated biosurfactant production and a measurable reduction in crude oil degradation, while Isolate 2 showed no significant change, indicating a lack of degradation activity. After 28 days of incubation in seawater broth enriched with 1% crude oil, Isolate 1 exhibited varying levels of crude oil degradation at different concentrations (0.4 mL to 1.0 mL). At higher concentrations, bacterial growth remained robust, and a noticeable reduction in the crude oil layer was observed, although some residual oil remained at 1.0 mL, suggesting a slight decrease in degradation efficiency. These results highlight the potential of Isolate 1 as a bioremediation candidate for addressing crude oil contamination in marine environments. Further research is required to optimize environmental conditions for enhanced degradation and to explore the molecular mechanisms underlying crude oil biodegradation by this isolate.

Keywords: Marine bacteria, crude oil degradation, biosurfactant, bioremediation, oil contamination.

Introduction

The Earth's surface is covered by vast seas and waterways, which constitute approximately one-third of the planet and serve as critical habitats for a wide array of aquatic species (de Oliveira Soares *et al*., 2020). However, human activities have introduced a variety of pollutants into these marine environments, leading to significant disturbances in aquatic ecosystems (Häder *et al*., 2020). Although natural gas and oil are vital to the global energy mix, accounting for 50% of the world's primary energy since 2018 (Ritchie and Roser, 2019), their integration into ecosystems presents substantial risks to public health and the environment (Soares *et al*., 2020). Activities associated with offshore oil exploration, transportation, storage, and accidental maritime spills often result in crude oil contamination, which becomes a major pollutant in aquatic ecosystems (Bi *et al.,* 2011). Additionally, roads, as primary sources of pollution, contribute to the entry of crude oil into water bodies, further exacerbating ecological damage (Hassanshahian *et al*., 2020).

Marine ecosystems undergo natural changes on various timescales, ranging from hours to millennia, and across spatial scales, from meters to entire ocean basins. While oil pollution is a prominent contributor, other factors such as human disturbance, habitat alterations, additional forms of pollution, fishing, changes in predation patterns, and shifts in weather and climate also drive ecological changes. The impact of oil on marine environments spans timescales from days to decades, depending on the severity of the spill, with chronic pollution affecting ecosystems over extended periods. Oil spills can impact the ocean across spatial scales, from tens of square meters to thousands of square kilometers, while chronic oil contamination may affect areas ranging from a few square centimeters to several thousand square kilometers. The biological effects of oil pollution are generally categorized as acute or chronic, with spills typically causing immediate, short-term damage due to high concentrations of petroleum. In contrast, chronic pollution, such as that from urban runoff into coastal areas, can lead to prolonged, low-level exposure with continuous ecological consequences.

The degradation of oil in the environment is influenced by a range of factors, including its organic composition, concentration, environmental conditions, and the presence of microbial communities. A study examining the degradation of oil in contaminated soils found that the half-lives of polycyclic aromatic hydrocarbons (PAHs) with low molecular weights in soils containing 1–2% hydrocarbons ranged from 1.5 to 5.5 weeks, while in soils with 13–56% hydrocarbons, the half-lives ranged from 2.5 to 52 weeks (Roslund *et al.,* 2018). Several oil

pollution remediation technologies have been developed, including incineration, froth flotation, solvent extraction, physical methods, chemical leaching, chemical oxidation, and cement solidification. While these methods are relatively simple, efficient, and well-established, they often fail to address the root causes of pollution and can be costly and require extensive engineering (Cerqueda-García *et al.,* 2020). Emerging technologies, such as thermal desorption and electrical remediation, also have limitations, including high energy consumption and the need for harsh operating conditions.

Ultimately, most petroleum hydrocarbons in the environment are degraded or metabolized by indigenous bacteria, which utilize these hydrocarbons as an energy source and for carbon to support their growth and reproduction. This microbial activity also alleviates the physiological stress caused by the presence of hydrocarbons in the surrounding environment (Hazen *et al.,* 2010; Kleindienst *et al.,* 2015a). Advances in microbial biotechnology and high-throughput sequencing technologies, such as microfluidic techniques (Jiang *et al.,* 2016; Guerra *et al.,* 2018), have facilitated the screening and identification of functional microorganisms in petroleum-contaminated environments. Numerous studies have demonstrated that hydrocarbon-degrading bacteria thrive in oil-rich environments, such as oil spill sites and reservoirs (Hazen *et al*., 2010; Yang *et al.,* 2015). The abundance and effectiveness of these bacteria are closely correlated with the type of petroleum hydrocarbons present and the surrounding environmental factors (Fuentes *et al*., 2015; Varjani and Gnansounou, 2017).

Materials and Methodology

Sample Collection

A marine water sample contaminated with crude oil was collected from the Pamban, Rameswaram, following an oil spill from a nearby boat. The sample was carefully stored in a sterile container to prevent further contamination and transported to the microbiology laboratory at VMASCW, Ramanthapuram, in a thermos box for further analysis.

Isolation of bacteria

The contaminated marine water sample was subjected to serial dilution. Ten sterile test tubes were labeled from 10^{-1} to 10^{-10} and filled with 9.0 mL of deionized water. A 1.0 mL aliquot of the contaminated water sample was added to test tube 10^{-1}, and the mixture was vortexed for 2 to 3 minutes to ensure thorough suspension. Subsequently, 1.0 mL from the 10^{-1}

dilution was transferred to the test tube 10^{-2}, and the dilution procedure was repeated through successive test tubes up to a final dilution of 10^{-10}. 0.1 ml from each suspension was transferred into the nutrient agar plates and incubated. The desired colonies from these were isolated by following the streak plate technique and preserved in nutrient agar slants.

Identification of Bacteria

Starch agar demonstrates the hydrolytic activity of exoenzymes, with starch serving as the polysaccharide substrate. Lipids, such as triglycerides, are high-energy compounds degraded by extracellular enzymes called lipases (or esterases), which hydrolyze ester bonds, releasing glycerol and fatty acids.

The Triple Sugar Iron (TSI) agar test differentiates Enterobacteriaceae, gram-negative bacilli that ferment glucose and produce acid, from other gram-negative intestinal bacteria. It classifies Enterobacteriaceae groups based on their biochemical properties and enzymatic reactions, which are further analyzed through the IMViC tests (Indole, Methyl Red, Voges-Proskauer, and Citrate Utilization) (Cappuccino & Welsh, 2019). Isolated colonies were streaked onto the respective biochemical media and incubated for biochemical analysis.

Screening of Crude Oil Degradation

The selected colonies were inoculated into 50 mL of seawater broth enriched with 1% crude oil in a conical flask. The bacteria utilized the crude oil as a carbon source and grew continuously. Colonies from this initial screening were further tested for their ability to degrade crude oil at varying concentrations. In the subsequent screening phase, the selected colonies were inoculated into fresh seawater broth and incubated for three days. Afterward, crude oil was added to the inoculated media in concentrations of 0.4, 0.6, 0.8, and 1.0 mL to evaluate their degradation potential.

Analysis of the Potential of Degradation

After 28 days of incubation, the crude oil was extracted from the tubes. The extracted oil was allowed to dry completely to remove any residual biomass and media, ensuring a more accurate analysis. Once dried, the crude oil was weighed using an analytical balance. The degradation potential was then calculated based on the weight of the extracted crude oil.

Results and Discussion

Isolation and Identification

The isolated colonies were examined for colony characteristics, cell morphology, and staining properties. The results observed were as follows:

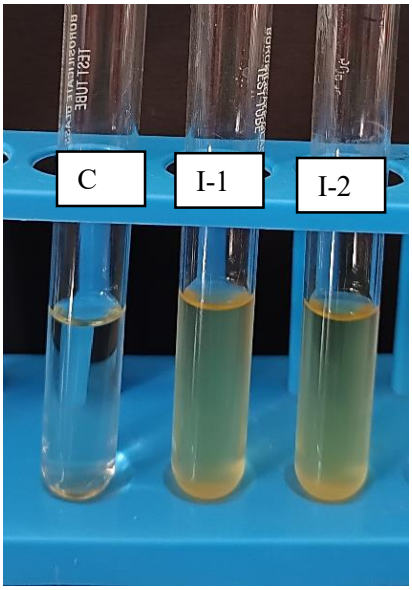

Fig 1: Isolates 1 and 2

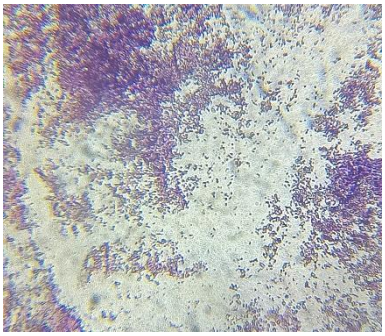

Fig 2: Gram Reaction of Isolate 1

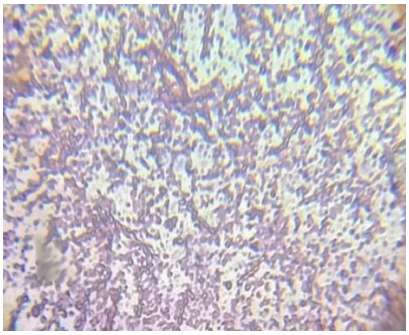

Fig 3: Gram Reaction of Isolate 2

Isolates	Colony Characteristics	Cell Morphology	Gram Staining Reactions
Isolate 1	Small, Round, White, Smooth, Opaque	Cocci, Chain, Small	Gram Negative
Isolate 2	Small, Round, White, Smooth, Opaque	Coccoid, Small	Gram-positive

Table 1: Cell Characteristics

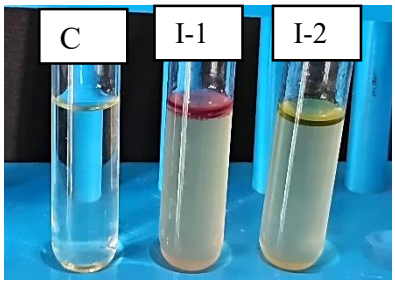

Fig 4: Indole Test

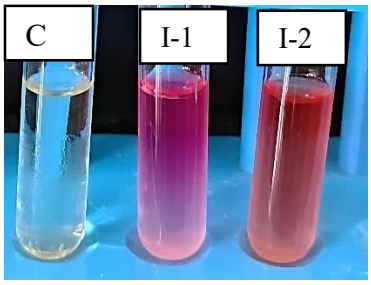

Fig 5: Methyl Red Test

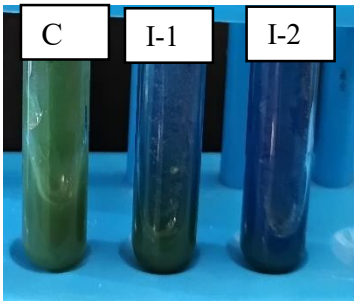

Fig 6: Citrate Test

Biochemical Test	**Isolate 1**	**Isolate 2**
Starch Hydrolysis	+	-
Lipid Hydrolysis	+	-
Indole test	+	-
Methyl Red Test	+	+
Voges Proskauer Test	-	-
Citrate test	+	+
Catalase test	+	-
Triple Sugar Iron Test	K/A	K/A H_2S, gas
Probable bacteria	*Aeromonas spp.*	*Micrococcus spp.*

Table 2: Biochemical Characteristics

Screening the efficacy of Crude Oil Degradation by the isolated Marine Bacteria

From the primary screening test, isolate 1, inoculated in seawater media enriched with 1% crude oil, showed biosurfactant production and a slight reduction in crude oil degradation. In contrast, isolate 2 did not exhibit any noticeable change in the crude oil layer, indicating no biosurfactant production or crude oil degradation. Based on these results, isolate 1 was selected for further testing with varying concentrations of crude oil.

After 28 days of incubation, the bacteria demonstrated varying degrees of crude oil degradation across different concentrations. At 0.4 mL, moderate degradation was observed, with further thinning of the oil layer and enhanced bacterial growth. At 0.6 mL, significant degradation occurred, with a more substantial reduction in the oil layer and robust bacterial growth, indicating effective breakdown at this concentration. At 0.8 mL, the degradation was even more pronounced, and active bacterial growth suggested efficient degradation at this

higher concentration. In the 1.0 mL crude oil tubes, bacterial growth remained substantial, and the oil layer showed considerable reduction, though some residual oil was still present, indicating that the bacteria could degrade at higher concentrations, but with slightly reduced efficiency. These results suggest that Isolate 1 has the potential for crude oil bioremediation, with significant degradation occurring at concentrations up to 0.8 mL, and some degradation still observed at 1.0 mL, though at a reduced rate.

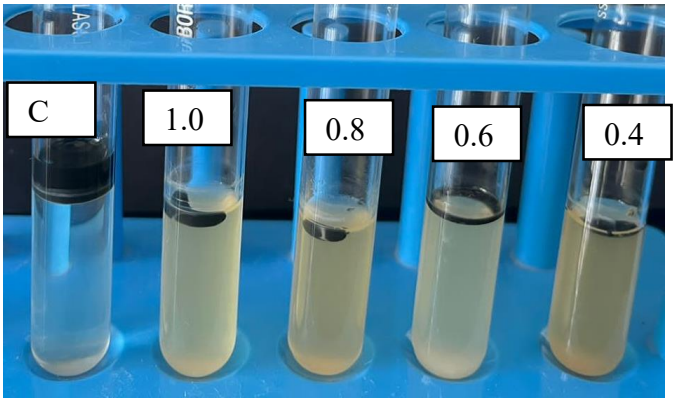

Fig 7: Screening of Crude Oil Degradation in Various Concentrations using Isolate 1

The degradation potential of the bacterial isolate was then calculated based on the difference in the weight of the extracted crude oil before and after incubation. This weight loss served as an indicator of the extent of crude oil degradation by the bacteria. The results were used to assess the efficiency of crude oil degradation at different concentrations and evaluate the isolate's potential for bioremediation applications.

Conclusion

This study successfully identified and evaluated two marine bacterial isolates for their potential in crude oil degradation. Isolate 1 *Aeromonas spp* demonstrated biosurfactant production and exhibited a measurable reduction in crude oil degradation, while Isolate 2 - *Micrococcus spp*. showed no observable change in the crude oil layer, indicating a lack of biosurfactant production and crude oil-degrading activity. Consequently, isolate 1 was selected for further screening and analysis. Upon incubation for 28 days, isolate 1 exhibited varying degrees of crude oil degradation across different concentrations. Initial degradation was observed at 0.4 mL crude oil, with increasing degradation observed at higher concentrations (0.6 mL and 0.8 mL). At the highest concentration of 1.0 mL, significant bacterial growth was still evident, and a notable reduction in the crude oil layer was recorded, albeit with some

residual oil remaining. These findings suggest that Isolate 1 is capable of effectively degrading crude oil, with a slight decrease in degradation efficiency at higher concentrations. The results of this study highlight the potential of Isolate 1 as a promising candidate for bioremediation applications aimed at mitigating crude oil contamination in marine environments. Further research is needed to optimize the environmental conditions for maximal degradation efficiency and to explore the molecular mechanisms driving crude oil degradation by this isolate.

Bibliography

Komal Sharma, Garishma Shah, Khushbu Singhal, Vineet Soni (2024) Comprehensive insights into the impact of oil pollution on the environment. *Regional Studies in Marine Science*, 74, 103516.

Shipra Jha, Praveen Dahiya (2022) Impact analysis of oil pollution on environment, marine, and soil communities. *Advances in Soil Water Separation,* 99 - 113.

John W. Farrington (2014) Oil Pollution in the Marine Environment II: Fates and Effects of Oil Spills, 6 - 31.

Cheng-Ying Jiang, Libing Dong, Jian-Kang Zhao, Xiaofang Hu, Chaohua Shen, Yuxin Qiao , Xinyue Zhang , Yapei Wang , Rustem F Ismagilov , Shuang-Jiang Liu , Wenbin Du (2016) High throughput single cell cultivation on microfluidic streak plates. *Appl. Environ. Microbiol,* 82 (7) 2210 - 2218.

Fuentes S., Barra B., Caporaso J. G., Seeger M. (2016). From rare to dominant: a fine-tuned soil bacterial bloom during petroleum hydrocarbon bioremediation. *Appl. Environ. Microbiol,* 82 (3) 888 - 896.

James G. Cappuccino, Emeritus, Chad Welsh (2019) *Microbiology, A Laboratory Manual*, 12,1-88.

Mehdi Hassanshahian, Giti Emtiazi, Simone Cappello (2012) Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea,7-12.

R. Anjali, Dr. K. Jagathy, Ms. P. Manjari (2018) Isolation and Characterization of Crude Oil Degrading Bacteria -*Bacillus sp*, 85 - 93.

Marcelo de Oliveira Soares, Eliana Matos, Caroline Lucas, Lucia Rizzo, Louise Allcock, Sergio Rossi (2020) Microplastics in corals: An emergent threat, 161,1-7.

João M. Pereira, Marina Carreiro-Silva, Anaïs Sire de Vilar, António Godinho, Ceri Lewis b, Christopher K. Pham (2024) Cold-water octocoral interactions with microplastics under laboratory conditions, , Deep Sea Research Part I – Oceanographic Research Papers, 213.

Donat-P Häder, Anastazia T Banaszak, Virginia E Villafañe, Maite A Narvarte, Raúl A González, E Walter Helbling (2020) Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications, 5:713:136586.

Mirunalini Ganesan, Ravi Mani, Sakthinarenderan Sai, Govindaraju Kasivelu, Mukesh Kumar Awasthi, Rajinikanth Rajagopal, Nur Izyan Wan Azelee, P.K. Selvi, Soo Woong Chang, Balasubramani Ravindran (2022) Bioremediation by oil degrading marine bacteria: An overview of supplements and pathways in key processes, Vol - 303,134956.

Thenmozhi M, Gayathri R, Varalakshmi V, Murugeswari T, Naveena J and P. Dhasarathan (2022) Isolation And Characterization Of Marine Bacteria For Crude Oil Degradation, 21(5), 768 - 776.

Bassim Al Khirbash, Moheeb Abdu Al Salehi, Hagib A Al-Hagibi and Nabil A. Al-Shwaf, Screening and Identification of Petroleum-Degradation Bacteria in the Surface Seawater from the Red Sea Coast of Al-Hodeidah City, Yemen, 2023, 1-6.

M.Srimathi1, M. Suganthi, S. Sugitha, K. Ashok Kumar, C. Ramprasath, G. Abirami (2024) Characterization of Crude Oil Degrading Marine Bacterium Bacillus licheniformis, *Indian Journal of Microbiology* , 64(4), 1697-1709.

R.Vignesh, A.Arularasan, V.Gandhiraj, R.Charu Deepika (2016) Isolation Identification And Characterization Of Potential Oil Degrading Bacteria From Oil Contaminated Sites, *International Research Journal of Engineering and Technology* 3 (4), 2503-2508.

Xingjian Xu , Wenming Liu, Shuhua Tian, Wei Wang, Qige Qi, Pan Jiang, Xinmei Gao, Fengjiao L, Haiyan Li, Hongwen Yu1 (2018) Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis, *Frontiers in Microbiology*, 9.

Mehdi Hassanshahian, Hamid Tebyanian, Simone Cappello (2012) Isolation and characterization of two crude oil-degrading yeast strains, Yarrowia lipolytica PG-20 and PG-32, from the Persian Gulf, 64(7):1386-1391.

Marja I. Roslund, Mira Grönroos, Anna-Lea Rantalainen, Ari Jumpponen, Martin Romantschuk, Anirudra Parajuli, Heikki Hyöty, Olli Laitinen and Aki Sinkkonen, (2018) Half-lives of PAHs and temporal microbiota changes in commonly used urban landscaping materials, *Peer J*. 19 (6), 4508.