



Isolation, Identification, and characterization of oil degrading bacteria from Satara S.T Stand workshop

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ABSTRACT

Bacteria that break down petroleum hydrocarbons are common in nature and can use these substances as sources of carbon and energy. But no single organism can break down all the constituents of crude oil or other fuels spilled into the ecological habitat, though some bacterial strains are able to convert oil into carbon dioxide and water. With the ongoing rise in demand for oil and other oil-related products, petroleum hydrocarbon pollution is getting worse. Due to their severe negative effects on both terrestrial and aquatic environments, these forms of pollution have received more and more attention. Major problems caused by oil contamination include pollution, disruption of wildlife, acceleration of global warming, and destruction to general locations. On the basis of morphological characteristics, Gram nature, and biological test findings, two distinct strains of oil-degrading bacteria were isolated on nutrient agar plates that are capable of degrading oil. The strains were noticeably different from one another. Our present research work is removed oil degrading traces.

Keywords: *Crude oil, Bacteria, Contamination, Hydrocarbons.*

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INTRODUCTION

The natural impact of the environment causes a variety of significant factors that affect the processing of crude oils and solid waste as well as the refining process [1]. Petroleum waste and hydrocarbons are frequently subjected to bioremediation processes at latitude scales over the world to lessen crude dispersion. The majority of gasoline and crude oil contains a variety of aromatic compounds, including hydrocarbons, which were employed to kill microorganisms found in water-soluble compounds and soil manure [6]. Utilizing different strains of bacteria and fungus, bioremediation is a technique used to remediate environmental contamination[2]. According to the current situation, pollution is rising due to petroleum-related factors, which increases the risk of water and soil pollution[3]. Petroleum products contain substances that are difficult for nature to break down naturally since they contain aromatic compounds. Industrial waste that contains petroleum products and other compounds that are lubricating induces services The solutions to these types of issues involve using bioremediation processes. Condition in hazards is primarily caused by sea level rise. In this method, environmental contamination is controlled by using microorganisms and fungi. Degrading microorganisms can have the same effects as powerful degraders[7]. According to certain investigations, *Acinetobacter*, and *Nocardia* are the relatives of the oil-degrading bacteria. Leocarbon-degrading bacteria that produce hydrocarbons are found in sediments close to hydrothermal occurrences [1]. The most pervasive environmental pollutant in the human environment is thought to be crude oil and its derivatives[3]. The ability to destroy the material in a cold, deep marine habitat, particularly the ability to aggregate oil droplets into flocks and to decompose hydrogen. Firmicutes strains with the potential to break down oil were found to include the alk gene as well as the *Rhizobium*, *Rhodobacter*, *Enterococcus*, and *Bavarii coccus* strains [5]. Identification of these microorganisms is essential for comprehending the role of microorganisms in hydrocarbon transformation, especially the once capable in situ degradation[6]. It has long been believed that the surface aerobic breakdown of petroleum hydrocarbons is caused by the flow of oxy-heterotic nutrients and the water into a pond is required for the biodegradation of petroleum[7]. Extracellular polymeric compounds are primarily made up of protein and carbohydrates and are secreted by microorganisms, playing a major role in the formation of marine snow. Although many bacteria have been identified to break down oil [8].

MATERIAL AND METHODS

Sample collection:

A polythene bag and spatula were used to collect soil samples from an oil-contaminated site in Satara, S.T. stand workshop, and they were then transported to the applied microbiology lab at the Yashwantrao Chavan Institute of Science Satara (Autonomous).

Culture enrichment and isolation of Hydrogen degrading bacteria:

1 gm soil sample was serially diluted up to 10^{-7} in a sterile Bushnell-Hass broth flask with 0.1 ml sample from 10^{-7} and 1 ml crude oil. Then the broth was incubated at 37°C for 7 days in a shaking incubator. On a sterile nutritional agar medium, 0.1 ml of broth sample was spread at 37°C and incubated the plate for 24 hours. Two distinct colonies on a nutrient agar plate were counted. In order to see well-isolated colonies, loopful solutions were streaked over nutrient agar plates using the four-quadrant technique.

Identification of isolated colonies:

Morphological characteristics and biochemical assays and 16S rRNA sequencing were done to confirm the genus and species of the isolated bacterial strains.

RESULT

With the help of 16S rRNA, isolated strains *Bacillus horikoshii* and *Stenotrophomonas maltophilia* are able to degrade oil from oil-contaminated sites which leads to major serious problems.

Isolation of oil degradation bacteria: A crude oil-degrading bacteria was shown to have isolated colonies (Figures 1 & 2) on nutrient agar media plates after enrichment in Bushnell-Hass broth and incubation from oil-contaminated sites.



Figure 1. Isolated colony C1



Figure 2. Isolated colony C2

Gram Staining of isolated colonies C1 and C2:

Gram staining was performed to check the Gram's nature of isolated bacteria. Short rods for Gram-positive (figure 1) of C1 and Gram-negative (figure 2) of C2.

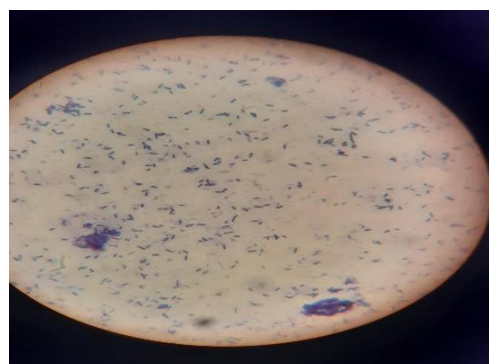


Figure 3: Gram staining of C1

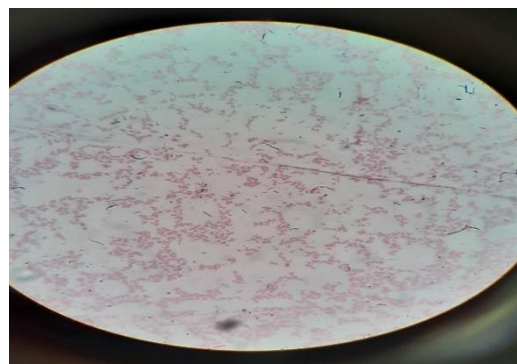


Figure 4: Gram staining of C2

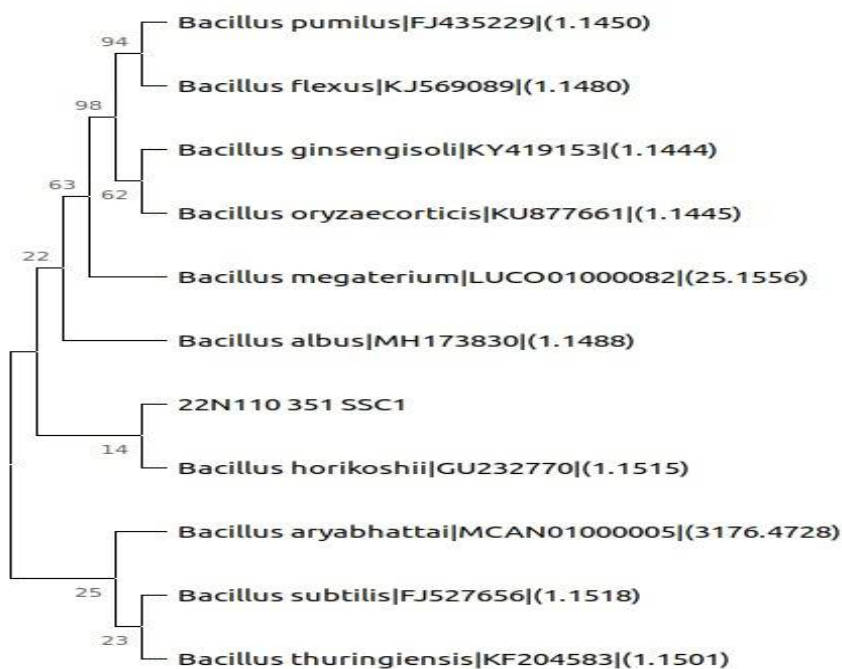
Table 1: Biochemical tests of C1

SrNo	Test	Result
1	Ribose	-
2	Xylose	-
3	Arabinose	-
4	Galactose	-
5	Sorbitol	-
6	Lactose	-
7	Maltose	+
8	Nitrate Reduction	-

Table 2: Biochemical test of C2

Sr.No	Test	Result
1	Citrate	+
2	Catalase	+
3	H ₂ S	-
4	Voges-Proskauer	-
5	Methyl Red	-
6	Nitrate Reduction	+
7	Glucose	-
8	Mannose	-
9	Mannitol	-
10	Arabinose	-
11	Galactose	-
12	Fructose	-
13	Lactose	-
14	Sucrose	+

Identification of isolated bacterial colonies by using 16S rRNA sequencing: 16S rRNA sequencing was used for identification of isolated bacterial colonies which were not confirmed by morphological and biochemical analysis. Isolated bacterial colonies were confirmed by 16S rRNA sequencing. Isolated colony C1 and C2 were identified as *Bacillus horikoshii* and *Stenotrophomonas maltophilia* respectively.

**Figure 5: Phylogenetic tree of C1 (*Bacillus horikoshii*)**

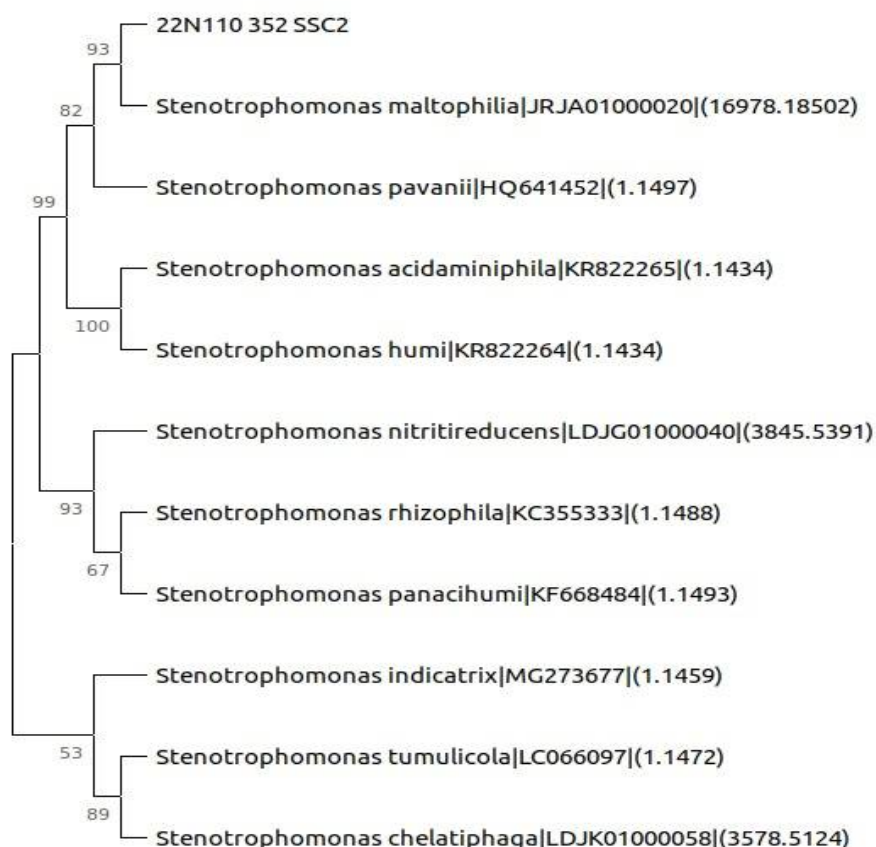


Figure 6: Phylogenetic tree of C1 (*Stenotrophomonas maltophilia*)

DISCUSSION

We sequenced *Bacillus horikoshii* and *Stenotrophomonas maltophilia* strain of crude oil degrading bacteria, isolated from Bushnell-Hass broth on nutrient agar. Sample collected from Satara S.T depot oil contaminated sites. *Bacillus horikoshii* strain which is one of the plays important roles in the genus bacillus has the ability to produce enzymes which not only oil degrading but alkali tolerant also[10]. There is enzyme-like hydrocarbon degradation present in crude oil degrading *Bacillus horikoshii* like methane monooxygenase, alkanemooxygenase, laccaseesterase, and lipase which are produced by bacillus Horikoshii for degradation of crude oil respectively[11]. *Stenotrophomonas maltophilia* is an emerging multi-drugresistant opportunistic pathogen that has an impact on community concern immunocompromised individuals. This is commonly found in aqueous habitats but we found it at oil-contaminatedsites in dry places of soil[12].

CONCLUSION

One of the most significant environmental problems is oil spills. Every year, there are thousands of oil leaks, and these spills in the water have grown to be a serious problem since they kill marine life and make seafood dangerous to eat. Leaks of oil and other petroleum. Oil degradation is the process of thermal breakdown of oil components present in sources of a contaminated site. In order to address these issues, it is necessary to find more potent strains of oil-degrading organisms. *Bacillus horikoshii* and *Stenotrophomonas maltophilia* are organisms that have the ability to degrade such toxic components which are responsible to cause pollution and contribute to the disruption of the environmental cycle. The use of *Bacillus horikoshii* and *Stenotrophomonas maltophilia* strains in the regulation of the enzyme system further degrades pollutants. Therefore, the work of this paper includes microbial degradation which is a key component of petroleum hydrocarbon remediation

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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