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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF AROMATIC HYDROCARBON DEGRADING BACTERIA FROM SOIL

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Abstract

Ten aromatic hydrocarbon degrading bacteria were isolated from petrol contaminated petrol pump sites in the Parbhani district. Based on a high growth rate on hydrocarbon degradation ability, three strains were selected from the ten isolated strains for further study. The 16S rRNA gene sequencing showed that these isolated strains belonged to genera Bacillus, Pseudomonas, & Staphylococcus. Among the three isolates, Pseudomonas strain was the most effective in degrading petrol. The results obtained in this study were indicates that Pseudomonas strain could degrade a wide range of hydrocarbons as well as some recalcitrant hydrocarbon components, and can be applied for bioremediation process.

Introduction:-

Petroleum oil, sometimes referred to as crude oil, is a complex blend of several components. Depending on the source of the oil, petroleum hydrocarbons (50–98%) make up the majority of crude oil, whereas alkenes account for 20–50% of it. Microorganisms including bacteria, yeast, fungus, and microalgae may breakdown petroleum hydrocarbons. fossil fuel hydrocarbons Because they require energy and carbon for growth and reproduction as well as to lessen physiological stress brought on by the presence of petroleum hydrocarbons in the microbial environment and native bacteria, bacteria play a significant role in the degradation of hydrocarbons. In fact, the majority of petroleum hydrocarbons are degraded in the environment by these bacteria. N-alkenes, cycloalkenes, and polycyclic aromatic hydrocarbons (PAHs) are oil hydrocarbons which are mostly widely spread as environmental pollutant. (Das and Chandran 2011).

The use of petroleum based products has increased over the years in the 21st century, predominantly for the purpose of transportation. This activity is essential in almost all industries, especially in manufacturing and agriculture. Even without major oil spills, small amount of hydrocarbons are being released into the nature during transport, distribution processes or other human activities. Petroleum hydrocarbons come in different forms such as lighter refined oil, petrol, kerosene, diesel, as well as heavy crude oil. These oils are complex mixture of other organic compounds such as aromatic cyclic hydrocarbons, complex branched aliphatic and cyclic alkenes as well as other residual substances. Some of these are termed recalcitrant because they do not degrade easily in nature despite being organic. These man-made substances have become persistent pollutants. (Kumar et al., 2011).

The ability of isolation of high number of certain oil degrading bacteria from petroleum contaminated soil. Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. Petroleum hydrocarbons can be divided into four classes Aromatic, saturates, Asphatenes

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(phenols, fatty acids, Ketones, etc.) and resins (pyridines, quinolones, amides etc.) One of the important factors that limit biodegradation of oil pollutants in environment is their limited availability to microorganisms. Microbial degradation is the major and ultimate natural mechanism by which cleanup the petroleum hydrocarbons pollutants from the environment. They studied the extensive biodegradation of alkyl aromatics in marine sediments which occurred prior to detectable biodegradation of alkane profile of the crude oil and the microorganisms namely, Bacillus, Mycobacterium, Pseudomonas, and Rhodococcus were found to be involved in degradation of petroleum hydrocarbons. (Jain et al.2011).

The main Factors which limit the overall biodegradation rate can be grouped as soil characteristics, contaminant characteristics, bioavailability, microorganism number and catabolism evolution. In order to adapt and employment some bioremediation strategy it is extremely important to consider and understand those limiting factors. The presence of oil degrading microorganisms such as bacteria and fungi is not restricted to a particular ecosystem and has been found in the Arctic, Antarctic and temperate region but little work has been reported in high temperate ecosystem. The ability of microorganism to utilize hydrocarbons in oil contaminated environments has been documented (Obuekwe et al., 2005).

Materials and Methods:-

Collection of samples

Sampling For the isolation of petrol degrading bacteria, soil samples were collected from different petrol pump sites in Parbhani district. Soil samples were taken from 1 to 12 cm below the surface using a sterile knife. All the collected samples were transported in strile bags to the laboratory.

Isolation and Primary screening of petrol degrading bacteria

Bushnell Hass Mineral Salts (BHMS) medium was used for the isolation of hydrocarbon-degrading bacteria. BHMS media was supplemented with 1% (v/v) petrol as the sole carbon source. Aliquots of soil samples were added to Erlenmeyer flasks containing 100 ml of medium, and the flasks were incubated for 4 days at 30^0C on rotary shaker at 120 rpm. Then 5-ml aliquots were removed and placed in fresh medium. After a series of four further subcultures, inoculums from the flask were streaked out, and phenotypically different colonies on BHMS agar were purified. Phenotypically different colonies obtained from the plates were transferred to fresh medium with and without petrol to eliminate autotrophy and agar-utilizing bacteria. The procedure was repeated, and only isolates exhibiting pronounced growth on petrol were stored as agar slant cultures for further characterization (Chaillan et al., 2004; Hasanshahian and Emtiazi, 2008).

Secondary screening of petrol degrading bacteria

All the bacterial strains isolated through primary screening were again processed for secondary screening. Active culture of each bacterial isolate was transferred separately to 100ml of MSM medium with 2% petrol as sole carbon source & incubated on rotary shaker at 30^0 C with 120rpm agitation speed for 72-120 hrs of incubation. After incubation 5ml aliquots were removed aseptically from each flask & optical density was measured at 660nm. The bacterial cultures showing highest optical density were selected for further studies (Ebadi et al., 2021).

Identification of isolates

Identification of isolated bacterial cultures was done on the basis of its growth characteristics on differential media and biochemical properties such as Grams nature, motility, lactose fermentation, indole production, methyl red Voges proskauer (VP) reaction, citrate utilization, H_2S production, catalase and urease were performed according to Bergey's Manual of Systematic Bacteriology. Molecular identification Analysis of the 16S rRNA gene was performed for the taxonomic characterization of the isolated strains.

Results:-

Isolation and screening of hydrocarbon degrading bacteria

In the present study during the primary screening, total fifteen (15) hydrocarbon degrading bacterial strains were isolated from different soil samples collected from various petrol pumps in Parbhani district, Maharashtra. The results obtained in primary screening were indicated in Table 3.1, which indicates that more hydrocarbon degrading bacteria were isolated from Site II.

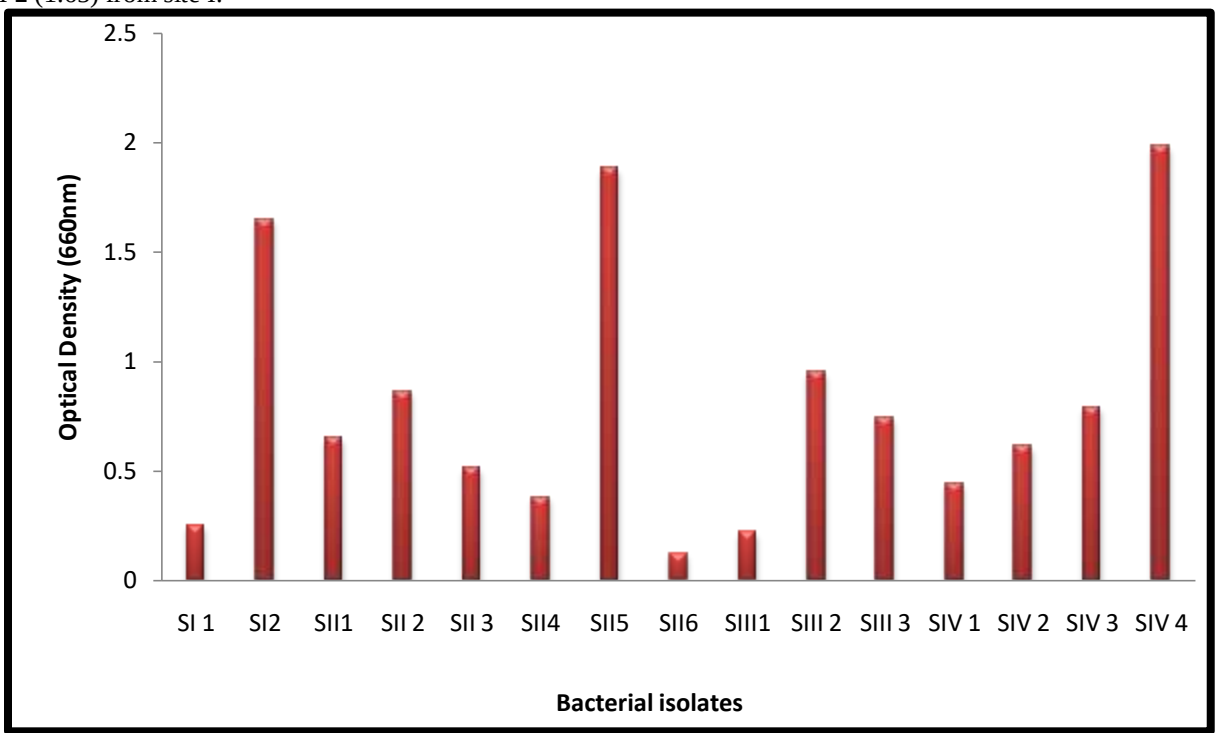
Table 1:- Number of isolates isolated from different sites in Parbhani District.

Sr.No.	Site of Collection	Number of isolates
1.	Site I	02
2.	Site II	06
3.	Site III	03
4.	Site IV	04

Secondary Screening of Petrol degrading bacteria

In the secondary screening all the isolated 15 petrol degrading bacterial isolates were evaluated for their degradation ability separately. In secondary screening three (3) potential strains with maximum petrol degrading ability were recorded on the basis of optical density measured at 660nm. The results obtained from secondary screening were represented in fig. 1.

From all 15 bacterial strains isolated from primary screening showed growth in the presence of petrol. The maximum optical density was showed by SIV 4 (1.98) isolated from site IV followed by SII 5 (1.88) from Site II & SI 2 (1.65) from site I.



Identification of Efficient petrol degrading bacteria

The efficient strains were identified on the basis of morphological and biochemical characterization (Table 1& 2.) according to Bergey's manual of bacteriology and further subjected to 16S rRNA gene sequencing. The Isolate MB-I (SII 5) was identified as Bacillus licheniformis with 100% homology, MB-II (SIV 4) as Pseudomonas stutzeri with 99% homology & MB-III (SI 2) as Staphylococcus hominis subsp. Novobiosepticus both with 100 % homology.

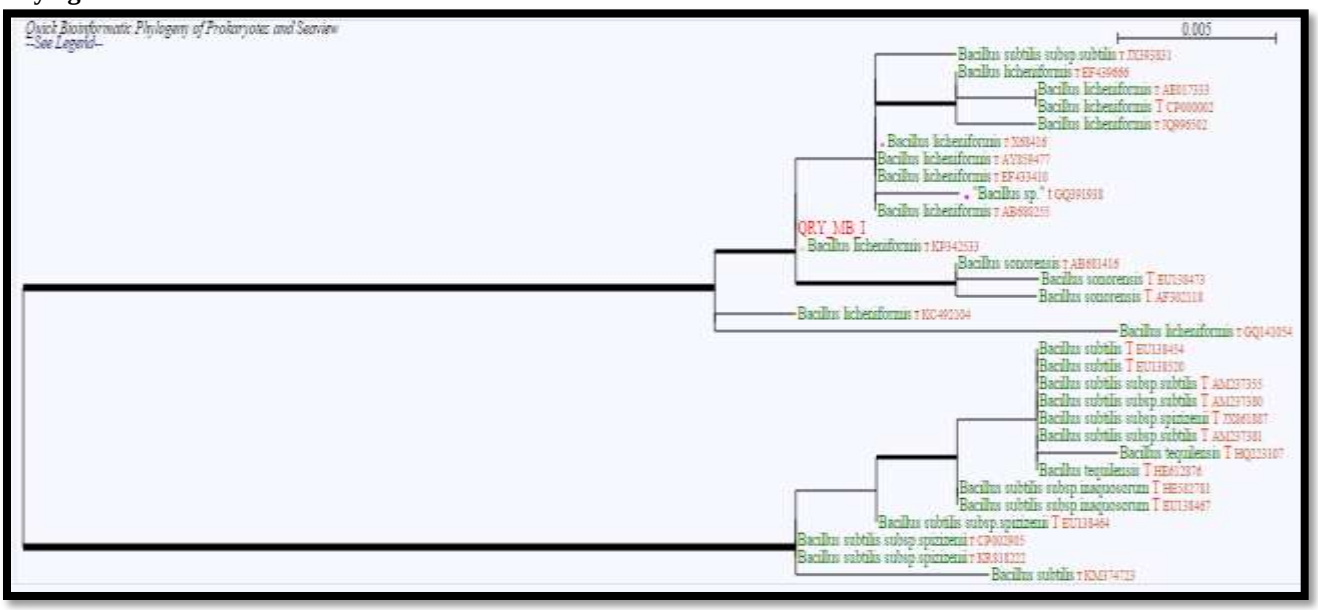
Table 2:- Morphological characterization of petrol degrading bacteria.

Character	**Isolate MB-I**	**Isolate II**	**Isolate III**
Shape	**Coccobacillary rods**	**Rods**	**Cocci**
Margin	**Irregular**	**Rhizoid**	**Entire**
Elevation	**Flat**	**raised**	**Elevated**
Color	**Cream**	**Cream**	**Yellow**
Appearance	**Sticky**	**Sticky**	**Sticky**
Grams Nature	**Positive**	**Negative**	**Positive**
Motility	**Actively motile**	**Actively motile**	**Non-motile**

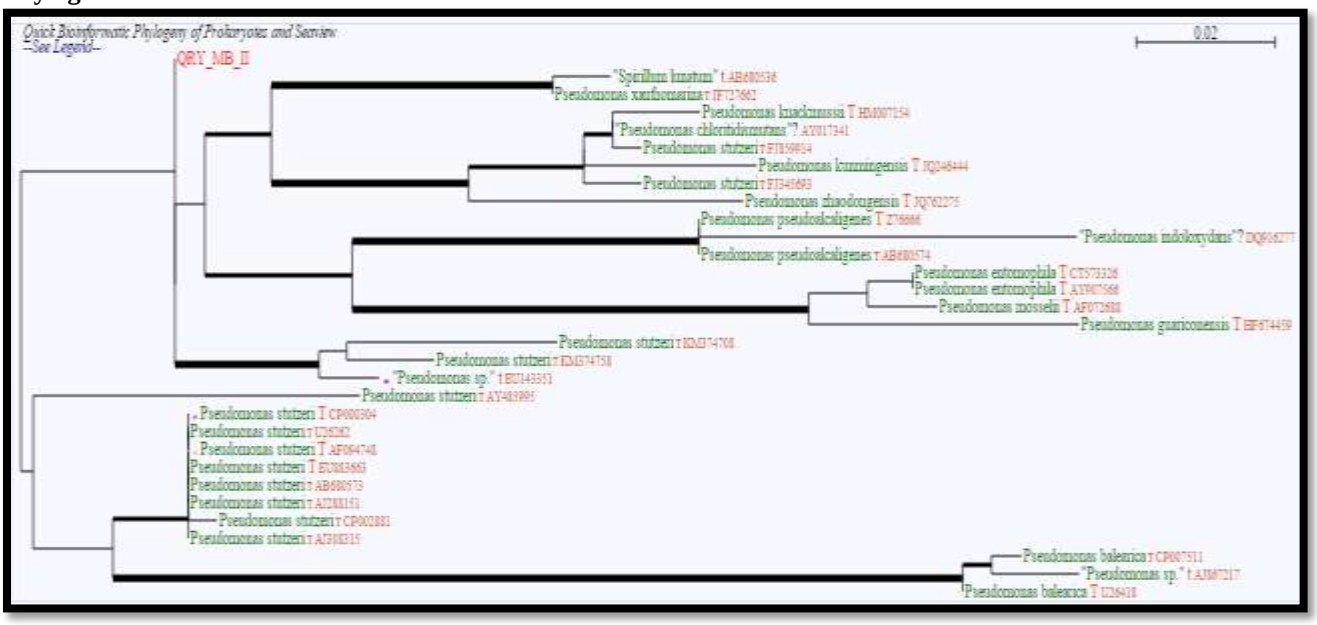
Table 3:- Biochemical characterization of petrol degrading bacteria.

Isolate	Indole	Methyl red	VP	Citrate	Catalase	Amylase	Oxidase	Nitrate reduction	H2S Production
MB-I	+	-	+	+	+	+	-	+	+
MB-II	-	+	-	+	-	-	-	+	-
MB-III	-	+	+	+	-	-	+	-	-

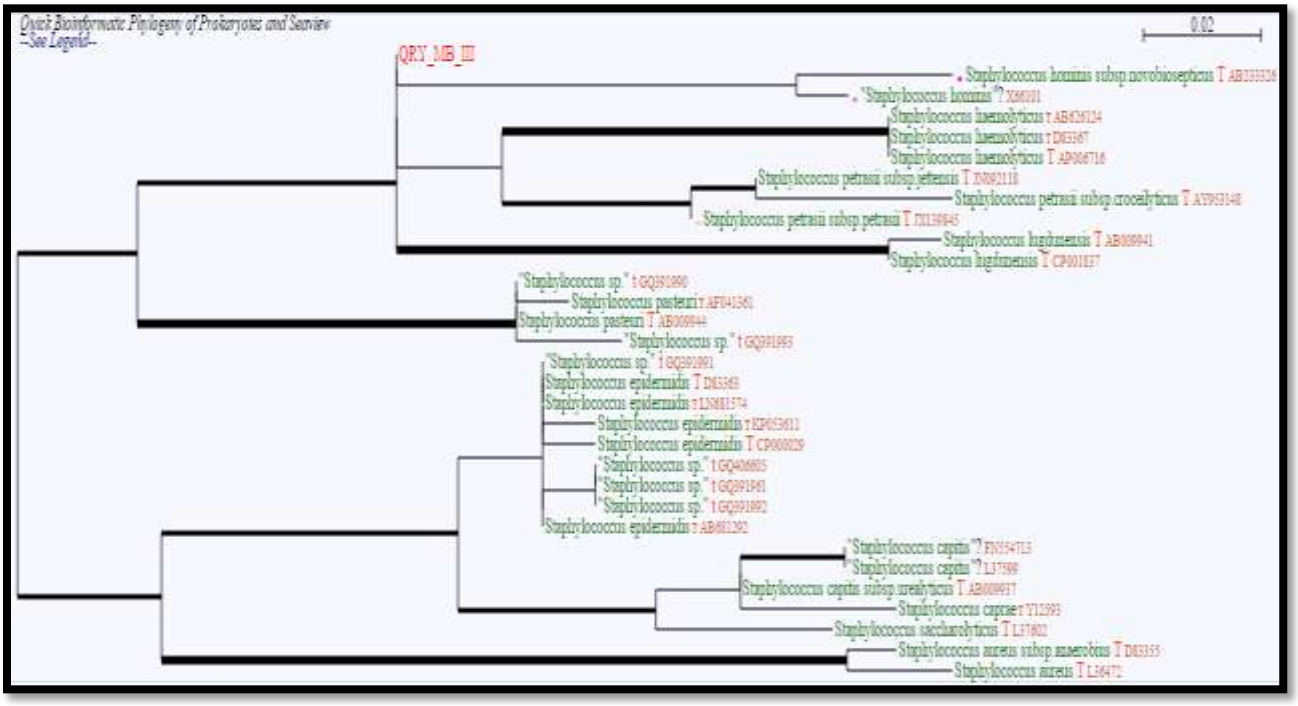
Phylogenetic tree of MB-I



Phylogenetic tree of MB-II



Phylogenetic tree of MB-III



Petroleum hydrocarbons are the most hazardous environmental pollutants, and will remain as the most important energy and chemical source as well as the most challenging organic pollutants in future (Peterson **et al.** 2003; Macaulay & Rees 2014; Khan **et al.** 2018). At present, a green alternative approach based on the principle of petroleum hydrocarbons degradation by indigenous or exogenous microorganisms to bioremediate pollutants and treat them safely is gradually emerging, which is very feasible and necessary for the current technological and social conditions (Frutos **et al.** 2012). Different kinds of potential strains have been isolated from the specific contaminated environment (Aurepatipan **et al.** 2018). For instance, eight potent degradative bacterial strains were enriched in the petroleum reservoir wastewater of Tehran and Kerman Provinces of Iran to separate alkane-degrading bacteria, with petroleum hydrocarbons as the sole source of carbon and energy (Hassanshahian **et al.** 2012).

In the present study, fifteen bacterial isolates were get isolated from petrol contaminated soil of Parbhani district. Three strains were found to degrade petrol very efficiently, indicating their significant potential in degrading petroleum hydrocarbons in soil. Some of the previous studies have suggested that bacterial strains can be used to degrade petroleum hydrocarbons. For instance, different hydrocarbons degradation rates for **Bacillus subtilis** were observed in liquid medium and soil after 14 days (38% and 30%, respectively) (Al-Dhabaan 2018).

Conclusion:-

Three bacterial strains presenting with highly efficient hydrocarbons degradation aptitude were isolated from heavy petrol contaminated soil in Parbhani district, and were identified as **Bacillus** licheniformis, **Pseudomonas** stutzeri & Staphylococcus hominis. Thus, the isolated strains have potential applications in the remediation of crude oil contaminated soil as well as treatment of oily sewage.

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