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Characterization of vegetable oil degrading bacteria from oil mill contaminated soil

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Abstract
Bioremediation method is considered to be more economical and safe method for the treatment of oil contaminated site. It has been observed that micro-organism that grows on oil contaminated soil is much capable of degrading oil than those microorganisms which are found on non-contaminated site of oil. This work was carried out to isolate microorganisms from vegetable oil-polluted sites and screen them for their lipolytic activity. The physicochemical properties of the oil contaminated soil samples were analyzed and the Microorganisms were isolated from oil mill soil samples contaminated with different types of vegetable oils such as neem oil, gingilly oil and coconut oil. The isolates were characterized, identified and screened for their oil degrading property. Those cultures, which showed oil degrading property, were used for the optimization of growth conditions by using broth dilution method and the zone of inhibition were identified by using different concentrations of vegetable oil samples. Hence, this study concluded that the selected bacterial strains which were isolated from the vegetable oil mill contaminated soil sample were more efficiently degrade the vegetable oil samples than the control sample.

Keywords: bioremediation; oil mill; vegetable oil; contaminated soil; bacterial strains

Introduction
Many microorganisms such as bacteria, yeasts, molds and a few protozoa are known to secrete lipases for the digestion of lipid materials. Vegetable oils contained fatty acids or triacylglycerols that may be broken down by bacteria, fungi and yeast. Lipase producers have been isolated mainly from soil or spoiled food material that contains vegetable oil. Vegetable oil spills are becoming more common and are potentially more challenging than hydro- carbon spills [1] and there is a need for microorganisms capable of degrading vegetable oil wastes[2]. Vegetable oils such as soybean oil, cotton seed oil and groundnut oil can be degraded by the bacterial isolates from vegetable oil contaminated sites and the cultures may be used to actively degrade the pollutants and detoxify the environment [3]. However, Bioremediation is an efficient, low cost and environmentally viable process to treat various contaminated sites. This process is based on the microbial potential to metabolize persistent compounds [4]. Microbes secrete various enzymes among them lipase which helps in degradation of oil [5] and also remediation of crude oil creates area of interest for research [6]. Hence, the aim of this study was to isolate the efficient bacteria capable of utilizing vegetable oil and that can be used for optimization study.

Materials and Methods
Collection of Samples
Vegetable oil contaminated soil samples were collected constituting filtrate obtained after oil extraction from Meenu vegetable Oil Mill, near Tiruchirappalli district, Tamil nadu, India. The samples were tested for the presence of vegetable oil degrading bacteria.

Isolation and Characterization
Vegetable oil degrading bacteria were isolated by using plating technique and the plates were incubated at room temperature. Bacterial colonies after Gram staining were transferred on Nutrient agar and incubated at room temperature for 24 hrs and Slants of pure cultures were also maintained. The colonies were characterized on the basis of their morphological and biochemical characteristics.

Optimization study
The effect of substrate concentration on the growth of bacterial isolates and their ability to degrade vegetable oil was determined using BH medium with pH 7.5 and supplemented with various concentration of vegetable oil at 37°C. The growth of bacterial isolates were determined spectro photo metrically at 600nm and expressed in terms of microbial biomass [7].

Results and Discussion
The bacterial isolates were obtained from the soil Contaminated with different types of vegetable oil and identified as *Pseudomonas* sp. Bacteria were the predominant organisms isolated from the samples [8]. The microbial isolates in this study proved capable of lipase production and indeed, the lipolytic activity has been observed for pure cultures [9]. The formation of opaque zones around the colonies of the selected isolates is an indication of lipase production by the organisms. The culture was identified by morphological and molecular basis as *Bacillus licheniformis* & *Bacillus pumilus* and the Presence of cellulase and protease enzyme may help in degradation study [10]. Microbial lipases occupy a place of prominence among biocatalysts are used for remediation of vegetable oil-polluted sites [1].
The samples from the subculture plate (Fig. 1) were chosen to be smeared onto microscope slides for the identification of bacterial morphology. Under the light microscope, the sample was observed as gram negative, rod-shaped morphology (Fig. 2) by the gram staining process. The

biochemical tests results indicated that the isolated bacterial sample was characterized as *Pseudomonas* sp. The higher percentage of microbial biomass was recorded in Bushnell-Haas medium supplemented with sunflower oil as carbon source and ammonium sulphate as nitrogen source.

Among the three bacterial isolates *Pseudomonas* sp was identified as efficient diesel oil degrader [7]. In the optimization study, different substrate concentration revealed that, 1% of different types of vegetable oil (Neem oil, gingelly oil and coconut oil) support excellent growth (Table 1). However, the bacterial strains *Pseudomonas* sp. was able to tolerate increased in concentration.

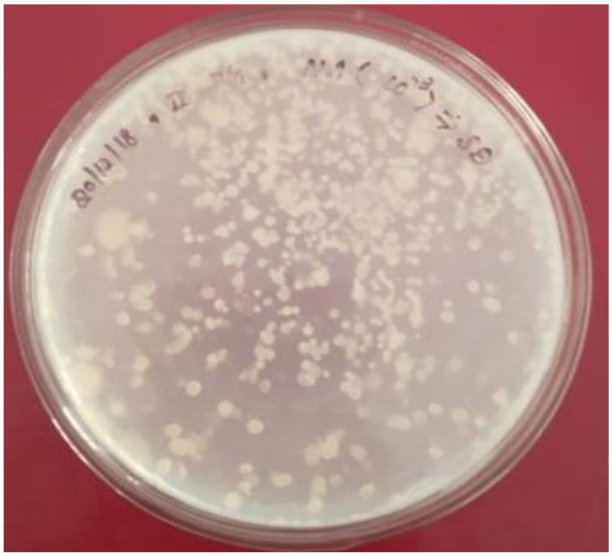

Fig 1: Bacterial isolates from oil mill contaminated soil sample

Fig 2: Microscopic view of *Pseudomonas* sp.

Table 1: Optimization study using broth dillution method

Dilutions	*Pseudomonas* sp	Coconut oil	Neem oil	Gingelly oil
10^{-1}	0.38	0.31	0.2	0.03
10^{-2}	0.31	0.39	0.27	0.05
10^{-3}	0.28	0.4	0.33	0.06
10^{-4}	0.20	0.43	0.38	0.08
10^{-5}	0.17	0.44	0.54	0.09

Conclusions

In conclusion, the obtained results showed that this isolate of *Pseudomonas* sp had high oil degrading efficiency. Therefore, we suggest that further research is done to determine the efficacy and viability of this isolate for longer periods in field or storage environments as a contact bioremediant in the area of oil mill comtaminated soil sample. Considering soil and environmental health, the enzymes obtained from *Pseudomonas* sp could be used as alternative biodegrading agents.

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References

1. Bukola Margaret Popoola A, Onilude A. Microorganisms Associated with Vegetable Oil Polluted Soil, Advances in Microbiology. 2017; 7(5):377-386.
2. Santhini K, Myla J, Sajani S, Usharani G. Screening of *Micrococcus* spp. From Oil Contaminated Soil with Reference to Biodegradation, Botany Research International. 2009; 2(4):248-252
3. Khan K, Naeem M, Arshed J, Asif M. Extraction and Characterization of Oil Degrading Bacteria, Journal of Applied Science. 2006; 6(10):2302-2306
4. Ivo Tamada S, Renato Montagnolli N, Paulo Lopes RM, Ederio Bidoia D. Toxicological evaluation of vegetable oils and biodiesel in soil during the biodegradation process, Braz. J. Microbiol. 2012; 43(4):1-6.
5. Veerapagu M, Sankara NA, Jeya KR, Alagendran S. Isolation and Identification of a Novel Lipase Producing Bacteria from Oil Spilled Soil, International Journal of Innovative Research in Science, Engineering and Technology. 2014; 3:18122-18129.
6. Guru GS, Gohel HR, Panchal MR, Ghosh SK, Braganza VB. Isolation and Enrichment of Microbes for Degradation of Crude Oil, International Journal of Engineering Science and Innovative Technology. 2013; 2:144-147.
7. Mahalingam PU, Nithya Sampath. Optimization of growth condition for diesel oil degrading bacterial strains. Advances in Applied Science Research. 2014; 5(6):91-96
8. Haliru M, Bukola CA. Screening of Microorganisms Isolated from Different Environmental Samples for Extracellular Lipase Production, AU J.T. 2012; 15:179-186.
9. Sharma RK, Jethani B, Raipuria M, Jain H. Antipyretic Activity of Aqueous and Alcoholic Extracts of Noni on Yeast Induced Pyrexia in Rats, International Journal of Pharmaceutical Sciences and Research. 2011; 2:1850-1854.
10. Gayatriben Patel B, Kamlesh Shah R. Biodegradation of cotton seed soap stocks by novel indigenous *Bacillus* species, Biosci. Biotech. Res. Comm. 2018; 11(3):505-511.