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A STUDY ON PROTEASE AND LIPASE PRODUCING BACTERIA FROM OIL CONTAMINATION SOIL AND SCREENING OF PYOCYANIN PIGMENT

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ABSTRACT

Oil spilled soil samples were collected from different places of Tamil Nadu such as Salem, Chennai, Kanchipuram, Vizhupuram and Vellore. The collected oil contaminated soil samples were used for isolation of protease and lipase producing Pseudomonas strains. Qualitative analysis of protease enzyme proteolytic zone was recorded in the oil spilled soil sample collected from Salem which recorded 3.0 cm followed by Villupuram, Vellore, Kanchipuram and Chennai. Qualitative analysis of lipase enzyme Lipolytic zone was recorded in the oil spilled soil sample collected from Salem followed by Vellore, Villupuram, Chennai and Kanchipuram. Quantitative assay of protease enzyme from five pseudomonas species were studied. Among five different places and five pseudomonas species, the maximum proteolytic activity was found maximum in pseudomonas speciesisolated from oil spilled soil sample collected from Salem followed by Villupuram, Vellore, Kanchipuram and Chennai. Quantitative assay of lipase enzyme from five pseudomonas species were studied. Among five different places and five pseudomonas species, the maximum lipolytic activity was found maximum in pseudomonas species isolated from oil spilled soil sample collected from Salem followed by Vellore, Villupuram, Chennai and Kanchipuram. Among five different places and five pseudomonas species were studied for pyocyanin pigment production. The maximum pyocyanin pigment production was found maximum in pseudomonas species isolated from oil spilled soil sample collected from Salem followed by Vellore, Kanchipuram, Villupuram and Chennai. Seed germination test: In this study, soil sample contaminated with oil and the same soil sample treated with the supernatant extracted from the isolated culture

of Pseudomonas species. Were used for the seed germination test. Two different Individual soil sample were used, one sample was oil spill contaminated soil and the other was treated oil spilled soil. Both of the were used for seed germination and growth studies. The clean surface sterilized seeds (25 number of green gram seed) were inoculated on oil spill contaminated soil and treated oil spilled soil. The sterile seeds were maculated into different petriplate, the maximum response of seed germination 85% and development of shoot length 5.3 m per plant were found in soil treated with enzyme solution followed by untreated 50. Thus to conclude that treated oil spill soil show more enzyme activity and excellent seed germination quality.

1.0 INTRODUCTION

Natural process such as volcanic eruptions and erosion, produce pollution, but the primary environmental concern today is with anthropogenic pollution. Since industrial revolution, human activities have produced pollution at a dramatically accelerating pace, and the pollution produced is increasingly toxic and persistent. "Man made" substances of extreme toxicity, such as pesticides, plastics, synthetic chemical products, and radioactive wastes represents an increasmg preponderance of the pollutants that are being released into the environment. While petroleum hydrocarbon serves as raw material for the manufacture of goods and services, which are symbols of our civilization, its fundamental components are sources of energy that human development is based. Crude oil and its products, though not man-made, but largely manipulated by man to satisfy, as well as to provide the ingredients for thousands of products we use every day. Oil spills affect many species of plants and animals in the environment, as well as humans (Plohl et al., 2002). Thus oil pollution has remained a considerable environmental problem. (Yakubu, 2007).

Therefore, despite decades of research, successful bio-remediation of petroleum hydrocarbon contaminated soil remains a challenge (Rahman *et al.*,2003). Both in situ and on -site treatment process by involving the use of microorganisms to break down hazardous organic environmental contaminants avoid the economic and technical disadvantages (Ahlert and Kosson,1983; Lee and Ward, l985, Kishore and Mukerjee,2006). The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment. Bioremediation is the most effective management tool to manage the polluted environment and recover contaminated soil. Bioremediation is an attractive and successful cleaning technique for polluted environment. The truth is that many crude oil spills

can persist within the environment for some time after the spill, breaking up and dissipating slowly and often requiring an active clean-up response. Microbial utilization of hydrocarbons is highly dependent on the chemical nature of the hydrocarbon compounds in petroleum mixtures and on the environmental conditions, as well as bacterial species (Atlas, 1995). Common oil degraders found in marine environments are Pseudomonas, Achromobacter, Flavobacterium, cynobacterVibn'o. Bacillus, Acetobacter. Norcardia Micrococcus, Corynebacterium, as well as fungi, algae and yeast (W atanabe, 2001).

2.0 MATERIALS AND METHODS

Collection of Oil Spilled Soil Samples: Oil spilled soil samples were collected from various districts like Salem, Chennai, Kancheepuram, Villupuram and Vellore. The collected oil contaminated soil samples were used for isolation of protease and lipase producing Pseudomonas strains. The collected sample was immediately transported to the laboratory and stored at 4°C in refrigerator for further studies.

Serial Dilution of Oil Spilled Soil Sample: 1 gram of soil samples was added to 9ml of phosphate buffer saline which is labeled as 10'l dilution solution was shaken vigorously for at least 1 minute. To suspend the organism in the solution and 1ml of 10'1 diluted solution was transferred in to a tube containing 9 ml of PBS using a sterile pipette. This tube was labeled as 10'2 dilution. Additional dilution like 103, 10"‘,10'5,10"‘,10'7,10'8 10'9 and 10"0 dilutions were prepared by repeating the process as described for initial dilutions for each of the collected oil contaminated soil sample. After incubation the number of colonies was counted and Colony Forming Unit Per ml was calculated in the samples of Oil spill contaminated soil and diary effluent.

3.0 RESULTS AND DISCUSSION

Qualitative Plate Assay for Protease Enzyme Activity: Maximum proteolytic zone was recorded in the oil spilled soil sample collected from Salem which recorded 3.0 cm followed by Villupuram (2 9cm) Vellorc (2.8cm), Kanchipuram (2.70m) and Chennai (2.6cm) (Table l) (Figure l) (Plate3).

Qualitative Plate Assay for lipolytic Enzyme Activity: Maximum lipolytic zone was recorded in the oil spilled soil sample collected from Salem which recorded 3.0 cm followed Vellore (2.8cm), Villupuram and Chennai (2.50m), and least in Kanchipuram (2.1cm) (Table 2) (Figure 2) (Plate4).

Quantitative analysis of proteolytic activity of protease enzyme: Quantitative assay of protease enzyme from five pseudomonas species were studied. Among five different places and five pseudomonas species, the maximum Proteolytic activity was found maximum in pseudomonas species isolated from 0il Spilled soil sample collected from Salem (326.7 U/ml) followed by Villupuram (254.1 U/ml, v 1) e lore (225.72 U/ml), Kanchipuram (176.66 U/ml) and Chennai (84.7 U/ml) (Table 3) (Figure 3).

Quantitative assay for Lipolytic activity of Lipase enzyme using olive oil as a Substrate: Maximum lipolytic zone was recorded in the oil spilled soil sample collected from Salem which recorded (3.0cm) followed by Vellore (2.8cm), Villupuram (2.50m), Chennai (2.5cm) and Kanchipuram (2.10m) (Table 4)(Figure 4).

Estimation of Pyocyanin: Maximum Pyocyanin pigment was recorded in Pseudomonas species isolated from the oil spilled soil sample collected from Salem which recorded 1.195 ug/ml followed by Vellore (0.853 ug/ml), Kanchipuram (0.512ug/ml). Villupuram (0.512 ug/ml) and Chennai (0.341 ug/ml) (Table 5) (Table 6) and (Table 6).(Figure 5).

Seed germination: Since soil samples from Salem recorded maximum pyocyanin pigmentproduction seed germination tests were carried out only in the soil samples fromSalem. The maximum response of seed germination 85% and development ofshoot length was 5.30m per plant were found in soil treated with enzyme solution followed by untreated soil. (Plate 5a,5b,6a,6b).

Table. 1. Qualitative analysis of protease enzyme activity.

S. No	Sample	Protease Zone (Cm)
1	Salem	3 cm
2	Chennai	2.6 cm
3	Kanchipuram	2.7cm
4	Villupuram	2.9 cm
5	Vellore	2.8 cm

Table. 2. Qualitative analysis of lipase enzyme activity.

S. No	Sample	Lipase Zone (Cm)
1	Salem	3cm
2	Chennai	2.5 cm
3	Kanchipuram	2.1cm
4	Villupuram	2.5cm
5	Vellore	2.8cm

Table. 3. Quantitative assay the protective activity of protease enzyme using casein as a substrate.

S. No	Sample	Protease activity U/Ml
1	Salem	326.7
2	Villupuram	254.1
3	Vellore	225.72
4	Kanchipuram	176.66
5	Chennai	84.7 U.

Table. 4. Quantitative assays for lipolytic activity pfemzyme using olive oil as a substrate.

S. No	Sample	Lipase Activity U/Ml
1	Salem	50 U/Ml
2	Vellore	41 U/Ml
3	Villupuram	37 U/Ml
4	Chennai	23 U/Ml
5	Kanchipuram	15 U/Ml

Table. 5. Estimation of pyocyanin.

S. No	Sample	Amount of Pyocyanin produced ug/Ml of culture
1	Salem	1.195 ug/Ml
2	Vellore	0.853 ug/Ml
3	Kanchipuram	0.512 ug/Ml
4	Villupuram	0.512 ug/Ml
5	Chennai	0.341 ug/Ml

Table. 6. consolidated table of the qualititative quantitative activity of protease and lipase with the amount of pyocyanin produced.

S. No	Sample	Protease zone(Cm)	Lipase Zone (cm)	Protealytic Activity U/Ml	Lipolytic Activity U/Ml	Amount of Pyocyanim produced UG/Ml of Culture
1	Salem	3cm	3 cm	326.7 U/ml	50 U/ml	1.195 UG/Ml
2	Chennai	2.6cm	2.5 cm	254.1 U/ml	41 U/ml	0.853 UG/Ml
3	Kanchipuram	2.7cm	2.1 cm	225.7 U/ml	37 U/ml	0.512 UG/Ml
4	Villupuram	2.9cm	2.5 cm	176.6 U/ml	23 U/ml	0.512 UG/Ml
5	Vellore	2.8cm	2.8 cm	84.7 U/ml	15 U/ml	0.341 UG/Ml

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