

Identification of Phenol Degrading Bacteria from Soil Collected From Palakkad

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Abstract: Phenol and its component are majorly toxic to the environment. It is hazardous when exposed to the organisms surface. Since Phenolic components are excessively sprinkled in nature, organisms including human beings are more exposed. The aim of the study deals with the collection of sample from Palakkad and analyse the ability of bacteria, which has the potential to degrade the hazardous Phenolic compounds. Majorly *Pseudomonas* species are found to be having the capability to degrade the Phenolic compounds and their derivatives. In this burgeoning population the soil is major factor which is having major contact to phenol and other toxic chemicals by chemigation etc. So the bacteria which has potential degrading ability from soil is identified.

Keywords: Phenolic compounds.

I. INTRODUCTION

Simple phenol is liquid or solid with low melting point, but its boiling point is high because of hydrogen bonds. Phenol is slightly solvable in water due to its ability to make hydrogen bounds with water (9 gram in 100 ml water) (Morrison and Boyd, 1992). In the current scenario there is a lot of anxiety regarding the use of chemicals such as selenium, mercury, nitrate, and most prominent being the Phenolic compounds and their derivatives. There are number of methods accessible for handling of phenol, biological handling is particularly attractive as it has likely to approximately involve in the degradation of phenol entirely by producing harmless last yield and least derivative dissipate production (Hill and Robinson, 1975). The toxicity of phenol has been widely analysed and their disastrous effect toward human and environment is greatly concerned. It causes negative effects to aquatic flora and fauna (Ghadhi & Sangodkar, 1995). Because of widespread occurrence of phenol in the environment many microorganisms utilizes phenol

as the sole carbon and energy source which includes both aerobic and anaerobic microorganisms. In future technologies for bioremediation, microbial systems might be the potential tools to deal with the environmental pollutants (Nair *et al*, 2008). Enzymes being biocatalyst, have major role in degrading the toxic compounds. By the biological degradation microorganisms and enzymes are capable to converting phenol into nontoxic intermediates of tricarboxylic acids via Ortho or Meta pathway (Powlowski and Shinglar, 1994). The vapor of phenol can be easily absorbed through the skin. Phenol in solution form, easily passes through the skin, and its metabolism occurs in the liver, although, it could occur in the lung and kidney too. Phenol is toxic in environment and could decrease enzymatic activity as well. Also, it is toxic to fishes and is mortal between 5 – 25 mg/l for them. It also deprives the fertility of the soil. Moreover, direct effect of phenol is a blocker for biologic reaction. Phenolic compounds are serious pollutant for rivers (EPA, 2004) and they have harmful effects such as growth inhibition, decrease of resistance against diseases, aquatic mortality and increase in growth of weedy plants. If phenolic pollution goes to underground water, it causes serious ecological problems.

II. MATERIALS AND METHODS

COLLECTION OF SOIL SAMPLE:

Soil was collected from the Palakkad district, the area from which soil is collected was more prone to toxic chemical effluents, majorly kerosene, which have a major portion of hydrocarbons which devast the environment.

MEDIA COMPOSITION AND CULTURAL CONDITIONS:

MSM media-composition: Sol A (400 ML)-NH₄NO₃-16g,KH₂PO₄-18.8g,NA₂HPO₄ -0.476,CaCl₂ -0.04g,ph-7,

Sol B (200ml) – MgSO₄.7H₂O-4g,MnSO₄.7H₂O-0.04g

Sol C (200ml) –FeSO₄ -0.03g

10ml of solution A mixed with 60 ml distilled water.10ml of solution B and 20 ml solution C was prepared in separate flask. All the three solution were sterilized separately by autoclaving at 121⁰C for 20 mins. Solutions were mixed prior to use.

Nutrient agar and Nutrient Broth media.

COLLECTION OF SOIL SAMPLE:

The soil sample was collected from Palakkad district, the soil was collected from kerosene spilled area. A quantity of 1g of soil sample was suspended in 100ml of MSM (Minimal Salt Media).

METHODS OF ISOLATING PHENOL DEGRADING BACTERIA:

10mg/L of phenol was used as sole source of carbon and then incubated in 250 ml flask at 37⁰C in rotary shaking incubator at 120 rpm for a week. A volume of 15 ml of enriched media was transferred into freshly prepared nutrient broth supplemented with phenol. A loopful of culture was streaked into the nutrient agar plates supplemented with phenol and incubated at room temperature. Single colonies were selected and inoculated into freshly prepared nutrient broth for preliminary identification .

III. RESULT AND DISCUSSION

The initial observation of phenol degrading bacteria, by collecting the soil sample and treating it with MSM and with phenol as a carbon source was carried out. Five isolates were obtained after streaking the culture in Nutrient agar media and the isolates were named as I1,I2,I3,I4,I5 and for preliminary identification gram staining was carried out. Among the isolated bacteria *Pseudomonas fluorescence* was an evident species, because of its unique morphological characteristics (Whiteley et al.,2001;)

Categorizations of isolated bacteria based on colony morphology

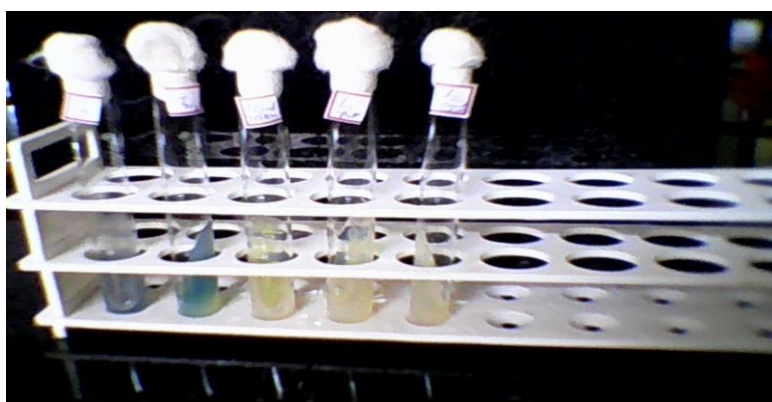
SL NO.	Name of Isolate	Size	Shape	Colour	Margin	Surface	Elevation	Transparency	Viscosity
1	I1	Small		White	Entire	Finely granular	Flat ingrowing	Opaque	Dry
2	I2	Large	Undulate	Light white	Irregular		Flat ingrowing	Transparent	Moist
3	I3	Small	Circular	Yellow	Entire	Finely granular	Flat	Opaque	Moist
4	I4	Large	Circular	Fluorescent green	Entire	Glistening	convex	Opaque	Moist
5	I5	Large	Circular	Fluorescent green	Entire	Glistening	convex	Opaque	Moist

Microscopic examination of selected colonies

ISOLATE	MICROSCOPIC FEATURES
I1	Gram positive,rod
I2	Gram negative,rod
I3	Gram negative ,rod
I4	Gram negative,rod
I5	Gram negative,rod



MSM MEDIA



Isolates identified from MSM Media-I1,I2,I3,I4,I5

IV. CONCLUSION

The phenol degrading bacteria was isolated. From the results observed from the isolates, *Pseudomonas fluorescense* is a promising organism obtained from the kerosene spilled soil from Palakkad District. Thus the future perspectives will be the biochemical and molecular level characterization of the isolates. Thus the isolates from the kerosene spilled region were identified.

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