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Screening and Characterization of Xenobiotic (Phenol) Degrading Bacteria with Reference to Bioremediation

KEYWORDS: Phenol degradability, Xenobiotic, microorganisms

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ABSTRACT *Microorganisms play key role for saving the environment by degrading various chemical compounds which are toxic in their native forms or may be modified either. Removal of such environment polluting compounds by microorganisms can be proved to be contemplatable. Because of their degradability, they are often found in the areas affected with such chemicals. And so the organisms can be isolated from the area. In the present study, we have focused for the degradation of the xenobiotic compounds and the organisms were isolated from the soil contaminated with oil by the enrichment technique using phenol as sole carbon and energy source, purified and checked for their ability to degrade phenol. The isolates were subjected to biochemical characterization at the genus level and according to Bergey's Manual of Determinative Bacteriology, the isolates characterized were Staphylococcus, Bacillus, Pseudomonas, Micrococcus, Streptococcus. Out of all the isolates, the third isolate Pseudomonas showed the best results for the phenol-degrading capability.*

INTRODUCTION

Environment gets contaminated with the waste we release into it. Some contaminants are degradable and some are not and hence retain in the environment. These contaminants are from many industries including petroleum, oil refineries, pharmaceuticals, etc. Out of all, the toxicity of phenol has been widely accepted and their ruinous effects towards human and environment is greatly concerned. It even causes negative effects to aquatic flora and fauna [1]. Phenol is poisonous even at a very low concentrations and the maximum limit for its tolerance for the environment is 1mg/litre. Taking up with the health issues, [2] deaths amongst adults have been reported with intake of phenol with the series starting 1.5 to 33g [3]. The volatile nature of phenol and its attraction to water can engender oral absorption of infected water which creates hazards to human being [3].There are number of methods easy to get to 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 [4]. It has been included in the EPA in 1979.

In future technologies for bioremediation, microbial systems might be the potential tools to deal with the environmental pollutants [5]. By the biological degradation microorganisms and enzymes are capable to converting phenol into nontoxic intermediates of tricarboxilic acids via Ortho or Meta pathway [6].

Biologically phenol degradation has been studied broadly and several investigations have reveal that phenol may be aerobically tarnished via a widespread diversity of microorganisms as well as pure bacterial culture like Acinetiobacter calcoaeceticus, Bacillus stearothermophillus, Burkholdaeria cepacea G4 [7-9]. +Alcaligines eutrophous, Nocardia species, Pseudomonas picketti, Pseudomonas putida Pseudomonas resinvorans and Ralstania eutrapha.

The nutritional requirements of microorganisms normally comprise nitrogen, phosphorus, potassium, sodium, calcium, magnesium, iron, trace elements and carbon. Dissolved oxygen is required for the respiration of the microbes under aerobic conditions. These requirements are fulfilled for the organisms through the media that comprises of phenol as the sole carbon source. As a result of this only those organisms will grow that uses phenol as sole carbon source. The increase in the growth of the organisms in the medium containing phenol indicates the consumption of phenol by them, their growth rate directly relates to the phenol degrading capability.

MATERIALS AND METHODS

Sample collection

The organism was isolated from soil. The soil sample was collected from the Ruchi Soya Industry area, Indore.

Isolation of bacterial strain by culture enrichment technique

A quantity of 1gm of soil sample was suspended in 100 ml of minimal salt medium containing Na_2HPO_4 (6g), KH_2PO_4 (3g), NaCl (0.5g), NH_4Cl (1g), $CaCl_2.2H_2O$ (1M) and $MgSO_4.7H_2O$ (1M) in 1000 ml double distilled water. 10mg/l of phenol was used as sole source of carbon and then incubated in 250ml flask at 37°C on rotary shaking incubator at 120 rpm for a week [10]. A volume of 5 ml of enriched media was transferred into freshly prepared media on each week supplemented with 10mg/l, and then incubated at 30°C.The single colonies were streaked onto nutrient agar plates, incubated at 30°C overnight and then the pure isolates were stored on LB agar slant supplemented with phenol as sole carbon source at 4°C until further use.

Identification of isolates

The isolates were identified based on morphological observation and biochemical characterization. The tests involved were gram staining, amylase and gelatinase production, citrate utilization, indole test, lactose, sucrose and glucose consumption, catalase test, oxidase test, nitrate reduction, etc [10]. Bergey's manual of determinative of bacteriology was used as reference to identify the isolates [11].

Strain selection of best phenol degrading capability

The isolates were coded as OSI (oil contaminated soil isolate) was inoculated into MSM (mineral salt medium) medium containing 10mg/l phenol as carbon source, for 48 hrs, and shaken at 120 rpm. After 48 hrs, the growth of cells was determined by turbidity measurement at 600 nm. The concentration of phenol was increased from 10mg/l to 90mg/l subsequently.

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Phenol removal and growth rate of phenol-degrading bacteria
2.5 mL of pure culture of isolates were added to the increasing concentration of phenol containing medium and inoculated for 24 hours. The phenol was determined quantitatively by spectrophotometric analysis using 4-aminoantipyrine as the color reagent [12] in the presence of an oxidizing agent in alkaline medium [13]. After incubation they were treated with 2 ml of 4-aminoantipyrine and 2 ml of potassium ferricyanide and O.D. was taken at 510 nm after 15 min. The optical density was calculated at 600 nm (OD_{600}) as bacterial growth rate [14].

RESULTS AND DISCUSSIONS
Bacterial isolation and identification
Soil contaminated with industrial oil was chosen as the source of indigenous microorganism isolation. High probability of the presence of toxic pollutants in this area was reason for the selection [15]. For one month, the sample was enriched in sterile MSM medium using phenol as sole source of carbon and energy. The sample was further treated with phenol to ensure that only phenol resistant strain would be selected.

After treatment, five bacterial strain isolates survived, and were identified as phenol degraders. The bacterial isolate coded as OSI-C have the best potential to phenol biodegradation based on high resistance of this xenobiotic compound. The bacterial isolates were morphologically and biochemically characterized and properties were listed in Table 1. The results for the tests were summed up and the organisms were identified at the genus level as per the Bergey's Manual of Determinative Bacteriology [16].

Table 1 : Morphological and Biochemical characterization of Isolates

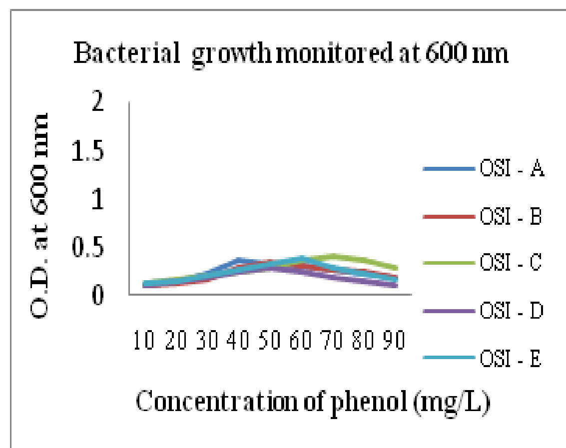
S. no.	Tests	Isolate 1 (OSI – A)	Isolate 2 (OSI – B)	Isolate 3 (OSI – C)	Isolate 4 (OSI – D)	Isolate 5 (OSI – E)
1.	Colonial characters	Circular, pin-point colonies, convex with entire margin	White, mucilaginous, irregular, translucent colonies, flat, serrate margin	Mucoid colonies, smooth margin, umbonate elevation	Yellow punctiform colonies, convex, entire margin	Yellow, slimy pin point colonies, circular entire margin
2.	Pigmentation	Golden yellow pigment	No pigment	Green pigment	Lemon yellow	No pigment
3.	Microscopic characters	Cocci, grape like structures	Rod shaped, chain like structures	Rod shaped, scattered cells	Cocci, tetrads	Cocci, chain structures
4.	Gram's staining	Gram Positive	Gram positive	Gram Negative	Gram positive	Gram positive
5.	Glucose fermentation	Positive	Positive	Negative	Negative	Positive
6.	Lactose fermentation	Positive	Negative	Negative	Negative	Positive
7.	Sucrose fermentation	Positive	Positive	Negative	Positive	Positive
8.	Indole production	Negative	Negative	Negative	Negative	Negative
9.	Methyl red test	Negative	Negative	Negative	Negative	Positive
10.	Voges-proskaeur test	Negative	Positive	Negative	Negative	Positive
11.	Citrate utilization	Positive	Negative	Positive	Positive	Positive
12.	H2S production	Negative	Negative	Negative	Negative	Positive
13.	Urease production	Positive	Positive	Negative	Negative	Positive
14.	Catalase test	Positive	Positive	Positive	Positive	Positive
15.	Oxidase test	Negative	Negative	Positive	Positive	Negative
16.	Lipid hydrolysis	Positive	Positive	Positive	Negative	Negative
17.	Starch hydrolysis	Negative	Positive	Negative	Negative	Positive
18.	Gelatin liquefaction	Negative	Positive	Positive	Positive	Positive
19.	Nitrate reduction	Negative	Positive	Positive	Negative	Negative

Table 2 : Organism identified from table 1 with reference to Bergey's Manual of determinative bacteriology

S.No.	Isolate	Organism
1.	Isolate 1 (OSI – A)	Staphylococcus
2.	Isolate 2 (OSI – B)	Bacillus
3.	Isolate 3 (OSI – C)	Pseudomonas
4.	Isolate 4 (OSI – D)	Micrococcus
5.	Isolate 5 (OSI – E)	Streptococcus

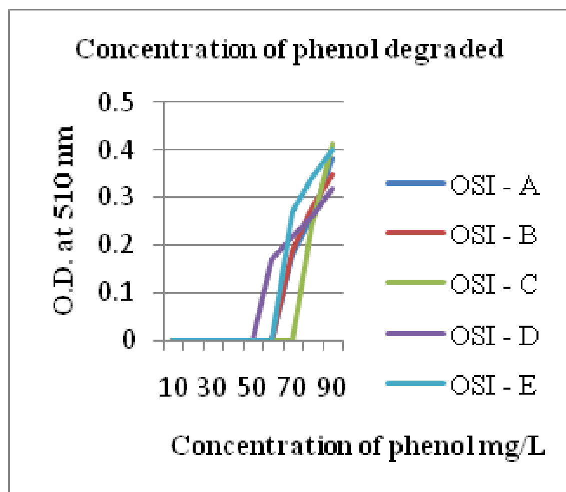
Phenol degradation studies
Bacterial isolate strains were grown in the Nutrient broth by incubation for overnight, at 37°C on shaker at 120rpm. This 24 hrs old culture was inoculated into MSM medium with phenol as sole carbon source. Preliminary degradation studies were carried out with addition of bacteria on media containing 10 mg/l of phenol. The reaction mixture contained all components but no bacterial inoculums were used as control. Then same procedure was followed by increasing concentration of phenol from 10mg/l to 90mg/l.

Figure 1: Bacterial growth in presence of phenol



To aliquot of standards, 2 mL of buffer solution was added and the pH should be 10 + 0.2. Then, 2.0 mL aminoantipyrine solution and 2.0 mL potassium ferricyanide solution was added and mixed.

Figure 2: Comparative graph for phenol degradation



The above chart shows that the maximum phenol degrading capacity is shown by the isolate OSI–C i.e., Pseudomonas spp. And it could utilize completely 60-70 mg/L phenol after 24 hours incubation. The phenol concentration was determined by analyzing samples after 48 hours of incubation by using UV-VIS spectrophotometer UV-1700. The residual amount of phenolic compound present in the sample was measured by colorimetric assay 4-amino antipyrine method [17, 18].

Conclusion

The organism, Pseudomonas can degrade the phenol into non toxic intermediates and hence is a useful candidate for bioremediation of polluted soil and water. The actual microbe xenobiotic interaction needs to be studied. The combined action of different species of bacteria will definitely prove to be more useful as compared to the single inoculants alone.

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