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Role of Pseudomonas Species in Bioremediation of Contaminated Soil

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ABSTRACT

The waste released into the environment contaminates the soil and 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. Indigenous microorganisms from the xenobiotic polluted soil are excellent tools for the bioremediation of such contaminated soils. Samples were collected from the contaminated soil and the organisms were isolated on the mineral salt medium with no carbon source except phenol. The potential of these isolates was tested for the degradation of phenol and the Pseudomonas species demonstrated the maximum capacity to degrade the phenols. The study was undertaken to observe the effects of in situ isolates on xenobiotic compounds like phenol and to know their effectiveness for bioremediation of such contaminated soils.

KEYWORDS : Phenol, bioremediation, contaminated soil, Pseudomonas

Introduction

Phenol is a toxic compound and its uncontrolled use in daily housecleaning process has increased its concentration in the environment. It is soluble in water and accumulates in soil hence is a xenobiotic compound. 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, (Calabrese, and Kenyon, 1991) deaths amongst adults have been reported with intake of phenol with the series starting 1.5 to 33g (Prpich and Daugulis, 2005). The best way to treat the soil is by use of microbial consortia which degrades phenol into intermediates like tri-carboxylic acids. The present study focuses only on single species of Pseudomonas where the growth rate and phenol degradability by this organism was studied. The pure culture of Pseudomonas strains are often utilized for metabolic pathway studies evaluating the degradation of many aromatic compounds such as phenol (Dowling and O'Gara, 1994, Timmis *et al*, 1994). In Pseudomonas, many induced enzymes are non-specific, and the metabolic pathway contains a high degree of convergences (Mahiudddin *et al*, 2012). The convergence of catabolic pathway allows for the efficient utilization of a wide range of growth substrates, while the non-specificity of the induced enzymes allows for the simultaneous utilization of several similar substrates without redundant genetic coding for enzyme induction (Hutchinson and Robinson, 1988).

Materials and Methods

Sample collection

The organism was isolated from soil. The soil samples were collected from the hydrocarbon contaminated soil from Indore area.

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 (Nagamani *et al,* 2009). 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 (Nagamani *et al*, 2009). Bergey's manual of determinative of bacteriology was used as reference to identify the isolates (MacFaddin, 2000).

Strain selection of best phenol degrading capability

The isolates were 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.

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 (APHA, 1995) in the presence of an oxidizing agent in alkaline medium (D. Svobodova and J. Gasparie, 1978). 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 (Saiqa et al., 1998).

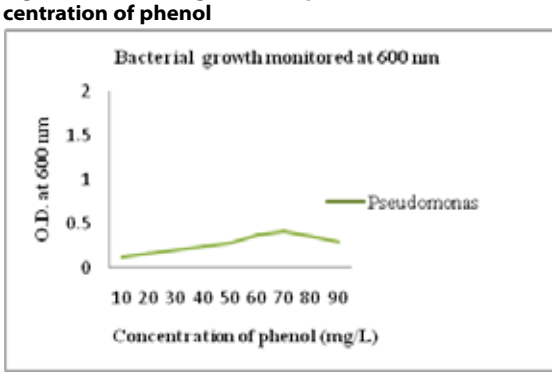
Results and discussion

The aim of the study was to isolate xenobiotic degrading bacteria and their effect on phenol removal. Soil contaminated with xenobiotics and sewage were the obvious choice of samples. The prominent isolates found in these samples were Pseudomonas. Their identification was based on the Bergey's Manual of Determinative Bacteriology (Bergey, D. H. (David Hendricks), 1860-1937; Breed, Robert S. (Robert Stanley), 1877-1956) and was identified as Pseudomonas aeruginosa. The colonial characteristics obtained were mucoid colonies, smooth margin, umbonate elevation with green diffusible pigment. Morphological studies demonstrate the organism to be gram negative, scattered small rods. The organism could utilize citrate, hydrolyse lipid, liquefy gelatin and reduce nitrate. The oxidase and catalase tests were also positive.

Phenol degradation studies

Bacterial isolate strains were grown in the Nutrient broth by incubation for overnight, at 37^{0}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 different concentration of phenol



Working standard for different concentrations of phenol

The stock solution was prepared by dissolving 1 gm phenol in 1000 ml distilled water. The standard was prepared with different concentrations of phenol as:

Table:1 Working standard for different concentrations of phenol

Working Solution (mL)	Concentration (mg/L)
10	10
20	20
30	30
40	40
50	50
60	60
70	70
80	80
90	90
100	100

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 Standard graph for phenol concentration

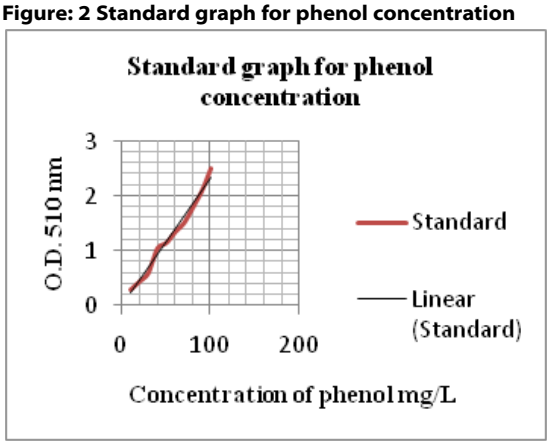
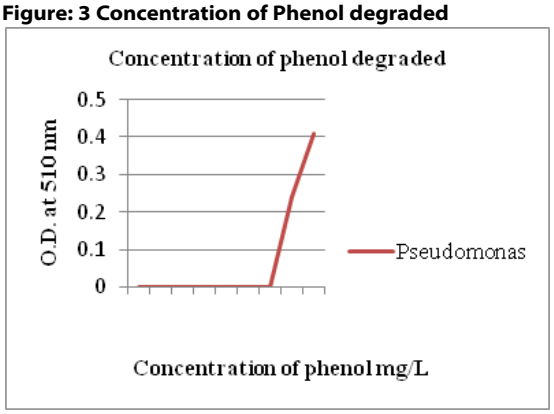


Figure: 3 Concentration of Phenol degraded



The organism could utilize 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 compounds present in the sample was measured by colorimetric assay 4-amino antipyrine method (Yang and Humphery, 1975; APHA, 1992).

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