

Evaluation of Phenol Biodegradation Potential of Indigenous Soil Isolate and Characterization of its Metabolic Pathway

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Abstract

Among all the aromatic compounds phenol is the major constituent of soil contaminated from many industries. Toxicity of phenol is well recognized for human as well as environment. In future, microbial system is the potential tool to deal with environmental pollutants like phenol. In the present study, an aerobic bacterial culture was isolated from oil contaminated soil by enrichment technique and screened for phenol degradation. The selected microbial strain was able to degrade phenol up to 200ppm. The isolated bacterial strain metabolized phenol via *meta* pathway which was confirmed by catechol 2, 3- dioxygenase enzyme assay. Outcome of this study offer a potential phenol degrader from the environment.

Keywords: Aromatic compounds, phenol, biodegradation, microbial systems.

Introduction

Phenol is a natural as well as man-made aromatic compound and one of the widely used organic compounds in existence. These have been reported as highly toxic and hazardous to living organisms. Due to their potential toxicity and persistence in the environment, rapid removal and detoxification is urgently needed¹.

Acclimatization of the microorganisms overcomes the substrate inhibition problems that normally occurred in phenol biodegradation at high concentration². Certain intracellular enzymes are induced during acclimatization stage so that the microbes are available to take part in the reaction³.

Phenol degrading aerobic bacteria are capable of converting phenol into non-toxic intermediates of tricarboxylic acid via *ortho* or *meta* pathway⁴. Catechol, a dihydroxy that substituted aromatic compound and key intermediate in the phenol

biodegradation, is cleaved by ring cleavage dioxygenases. Cleavage can occur either between two hydroxyl groups (*ortho*) or proximal to one of the two hydroxyl groups (*meta*)⁵.

The initial ring fission is catalysed by an *ortho* cleaving enzyme, catechol 1, 2 dioxygenase or by a *meta* cleaving enzyme catechol 2, 3 dioxygenase, where the product of ring fission is a cis-muconic acid for the former and 2-hydroxy cis muconic semi aldehyde for the latter⁶.

In this study, a phenol degrading strain was isolated and further characterization of its degradation pathway was done by studying the enzyme of degradation pathway.

Materials and methods

Isolation and Identification of strain

An aerobic bacterium was isolated by enrichment culture technique from oil contaminated soil samples in mineral salt medium⁷ containing 10 mg/l of phenol as sole source of carbon. After incubation period, the soil particles were allowed to settle and 5 ml of particulate free suspension was then used to inoculate a 100 ml minimal medium containing 10 mg phenol. Four such transfers were made and every time the enriched population was plated on minimal medium plates containing phenol as sole carbon source. After one month of enrichment, a pure isolate capable of growth on phenol was obtained.

The strain was identified based on morphological observation and biochemical characterization. Bergey's Manual of Determinative of Bacteriology was used as a reference to identify the strain⁸.

Phenol degradation studies

Phenol degradation ability of the strain was studied by culturing in MSM medium with different phenol concentrations ranging from 10 - 200 mg/l, for a period of 10 days at 37°C. For each experiment freshly prepared inoculum of O.D 0.1 was used. The samples were analyzed regularly for phenol degradation and the residual phenol concentrations were determined by 4-amino antipyrine method⁹. Growth behavior was monitored spectrophotometrically using UV spectrophotometer 166 Systronics at 600 nm by collecting the culture at regular interval for a period of 18h.

Characterization of metabolic pathway

In general aerobic metabolism of phenol is initiated by two different pathways either *ortho* or *meta* pathway. The presence of enzymes 1,2 dioxygenase (C12O) and 2,3dioxygenase (C23O) was characterized which gives an idea about phenol degradation pathway.

Enzyme assay

Preparation of enzyme extracts

Cells collected were washed twice with cold 100mM potassium phosphate buffer (pH 7.5) and resuspended in the same buffer. The cell suspension was disrupted by sonication. Unbroken cells and cell debris were removed by centrifugation

10000rpm for 10min at 4⁰C. The clear supernatant was used for enzyme assays. The protein concentration of cell extract was determined by Lowry method¹⁰.

Enzyme assays

Reaction mixture was prepared as follows containing 2ml EDTA (40mM), 13.8 ml phosphate buffer (50mM, pH 7.5), 0.2ml catechol (30mM) and 0.4ml cell free extract. Reaction was started by adding catechol. Activities of C12O and C23O were measured spectrophotometrically by monitoring the formation of reaction products at 260nm and 375nm respectively. The crude extracts used for C12O activity measurements were retreated for 5min with H₂O₂ (0.01%) to suppress the activity of C23O^{11, 12}.

Results and discussion

Isolation and characterization of phenol degrading strain

Isolation of phenol degrading bacterial strain was done from the soil sample collected near oil industry. Three isolates that were able to utilize phenol as sole source of carbon were obtained from the enriched population grown in MSM medium, supplemented with phenol. However, only one strain capable of utilizing phenol completely within 18 h was characterized using morphological and biochemical properties. By comparing these characteristics with Bergey's manual of systematic Bacteriology, the bacterium was identified as *Streptococcus spp.* It is a Gram positive, motile, cocci showing growth under aerobic conditions with the optimum temperature of 37°C.

Biodegradation of phenol

The time dependant changes of phenol concentrations in the media were evaluated. The bacterial strain was able to degrade phenol almost completely within a relatively short time. The phenol tolerance as well as ability to grow at elevated level of phenol concentration was investigated. The experiment aimed to find the highest tolerance level of phenol concentration found that it able to survive and degrade up to 200mg/l phenol. The growth of bacteria and phenol concentration in media showed the inverse proportion with each other. The strain showed a short period of lag phase for 3 h (Figure 1). When initial concentration of phenol was 200mg/l, complete utilization of phenol was within 18 h with a corresponding increase in cell biomass. The approximate doubling time (generation time) of the cell was 2 h, elucidated from the growth behavior of the strain.

Determination of metabolic pathway

In order to clarify the metabolic pathways of phenol, the activities of catechol 1, 2-dioxygenase and catechol 2, 3-dioxygenase were detected.

Aerobic and anaerobic catabolism of phenol in microorganisms is mediated by catechol dioxygenases. These are critical enzymes involved in the ring cleavage of catechol, during the biodegradation of phenol. To confirm the metabolic pathway enzyme assay for 1, 2-dioxygenase and 2, 3-dioxygenase was carried out (Ali et al,

1998). The cell free extract of the strain has shown catechol-2, 3- dioxygenase activity, indicating that the oxidation of catechol takes place through extradiol (*meta*) cleavage. Specific activity of catechol-1,2-dioxygenase was $0.112 \mu \text{ mole/min/mg protein}$. According to intermediates detection and key enzyme assay the metabolic route carried out in the strain was *meta* pathway.

Conclusion

The efficient phenol degrading bacteria was isolated and identified. The bacterial strain metabolized phenol by *meta* pathway of degradation. Hence it could be apply as potential phenol degrader to tackle the problem of it as environment contaminant.

Table 1: Specific activities of catabolic enzymes in crude extract of isolated strain.

Enzymes	Activity($\mu\text{mol min}^{-1} \text{ mg protein}^{-1}$)
C12O (Catechol -1,2- dioxygenase)	ND*
C23O (Catechol -2,3 - dioxygenase)	0.112

*ND- Not determined

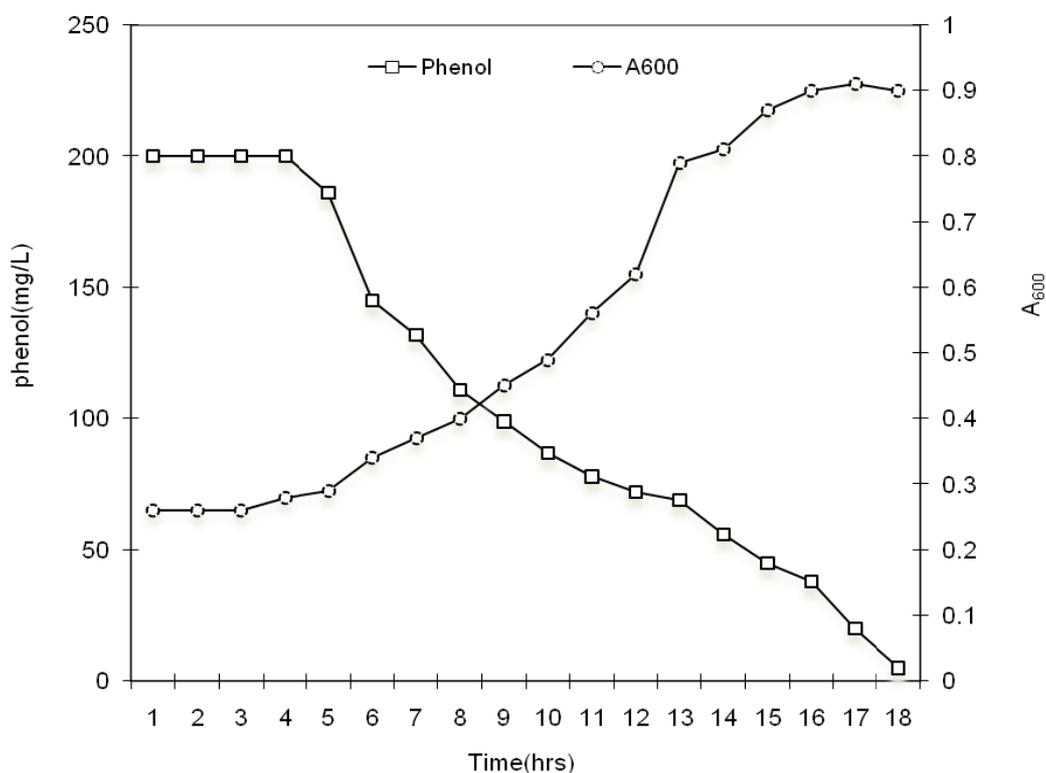


Figure 1: Growth behaviour of strain on phenol supplemented mineral salt media.

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