

# Isolation, Selection and Biodegradation Profile of Phenol Degrading Bacteria from Oil Contaminated Soil

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**Abstract** In the present study, an aerobic bacterial strains OCS-A and OCS- B were isolated from an oil contaminated soil. The strains were identified to be *Citrobacter freundii* and *Proteus mirabilis* according to morphological, physiological and biochemical characteristics. The strains were able to degrade about 90% of 100 mg/L phenol within 80 h as sole carbon and energy source. The lag phase increased with increase in phenol concentration. Determination of metabolic intermediate 2-HMS, was done which indicate *meta*-cleavage pathway of phenol *metabolism*. Hence these isolates can be effectively used for bioremediation of phenol contaminated sites.

**Keywords** Phenol · *Meta*-cleavage pathway · Bioremediation · Microbial *metabolism* · 2-HMS

## Introduction

Now a day's phenol is used by many industries in the manufacturing of products like insulation materials, adhesives, lacquers, paint, rubber, ink, dyes, illuminating gases, perfumes, soaps and toys. Phenol is one of the most widely used organic compounds in existence and is a basic structural unit for a variety of synthetic organic compounds including agricultural chemicals and pesticides, vegetables and coal. The German chemist, Runge isolated phenol from coal tar in 1834 and named karbolsaure (coal–oil acid or carboic acid), though its composition was not known until 1841 (Nair et al. 2008).

Biodegradation of phenol has increased strong attention throughout the world due to its poisonous properties and unrelenting nature (Leung et al. 1997). Microbial uptake and mineralization of phenol has been studied extensively (Dua et al. 2002; Lovely 2003; Watanabe 2001). The microorganisms that are normally used in phenol degradation include *Pseudomonas* sp, *Candida tropicalis*, *Azotobacter* sp, *Alcaligenes* sp, *Acinetobacter* sp etc. (Basha et al. 2010). The biological degradation is accomplished through benzene ring cleavage mediated by intracellular enzymatic reaction (Kumar et al. 2004).

This study was aimed to evaluate the potential of phenol degraders from environment. Phenol degrading bacteria were isolated and their performance in treating the compound was investigated.

## Materials and Methods

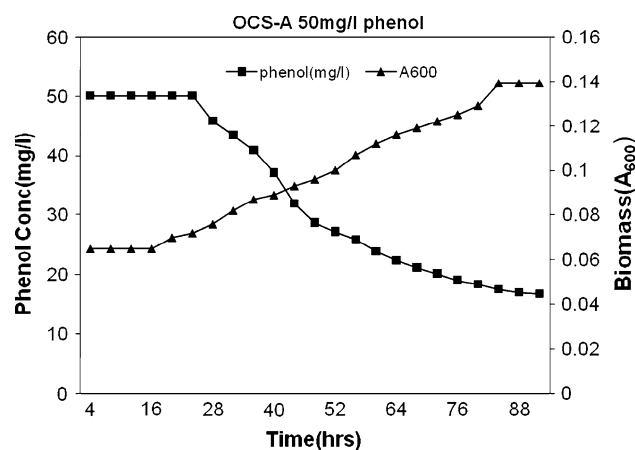
Two pure cultures used in this study were isolated from oil contaminated soil. The isolates were periodically subcultured on LB agar at 30°C and further maintained at 4°C. MP and MSM media were used in present study. MP medium composed of K<sub>2</sub>HPO<sub>4</sub>–2.75 gm, KH<sub>2</sub>PO<sub>4</sub>–2.25 gm (NH<sub>4</sub>)NO<sub>3</sub>–1.0 gm, MgCl<sub>2</sub>·6H<sub>2</sub>O–0.2 gm, NaCl–0.1 gm, FeCl<sub>3</sub>·6H<sub>2</sub>O–0.02 gm, CaCl<sub>2</sub>–0.01 gm (Watanabe et al. 1998). The minimal salt media (MSM) was used in this study. The medium was composed as: Na<sub>2</sub>HPO<sub>4</sub> (6 g), KH<sub>2</sub>PO<sub>4</sub> (3 g), NaCl (0.5 g), NH<sub>4</sub>Cl (1 g), CaCl<sub>2</sub>·2H<sub>2</sub>O (1 M) and MgSO<sub>4</sub>·7H<sub>2</sub>O (1 M) in 1,000 mL double distilled water (Nagamani et al. 2009). The medium was autoclaved at 121°C for 15 min for sterilization. Phenol (10 mg/L) was separately sterilized and aseptically added into the sterile medium.

Sample was collected from contaminated soil from oil industry and added into MP and MSM media in which

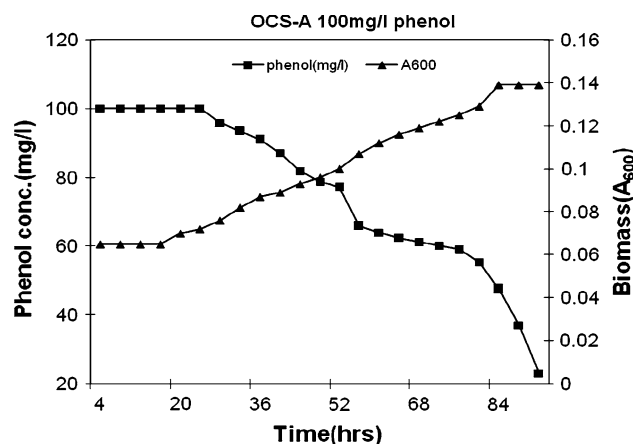
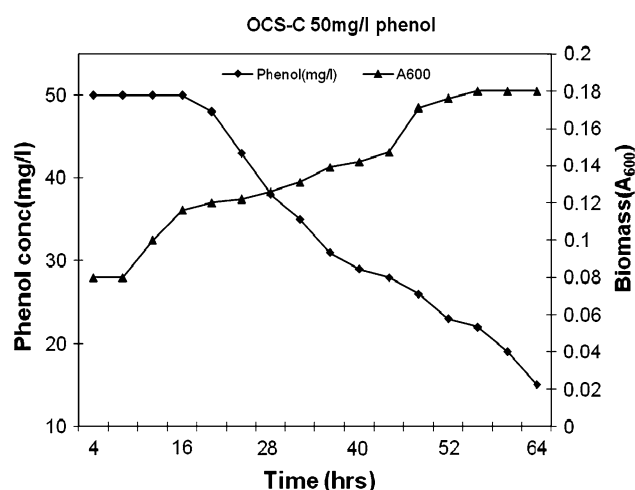
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**Table 1** Morphological and biochemical characteristics of bacterial strain OCS-A and OCS-C

Characteristics	OSC-A	OCS-C
Gram staining	Gram negative	Gram negative
Shape	Rod	Rod
Motility	Positive	Positive
Capsule staining	Capsulated	Capsulated
Acid Fast nature	Non Acid Fast	Non Acid Fast
Endospore	Terminal spore	Sub terminal spore
Oxygen Requirement	Aerobic	Aerobic
Colony	Pale yellow Slimy	Pale yellow Slimy
Catalase	Positive	Positive
Urease	Positive	Positive
Amylase	Positive	Positive
Gelatinase	Positive	Positive
Pectinase	Positive	Positive
H <sub>2</sub> S production	Positive	Negative
Nitrate reduction	Negative	Negative
Indole Production	Negative	Negative
Methyl red test	Positive	Negative
Vogus Proskaur Test	Negative	Negative
Citrate Utilization	Positive	Positive
Maltose	Positive	Positive
Lactose	Positive	Negative
Sucrose	Positive	Positive
Fructose	Positive	Positive
Galactose	Negative	Negative

**Fig. 1** Degradation profile by OCS-A of 50 mg/L phenol

phenol (10 mg/L) was added as sole carbon source. It was incubated for 24 h at room temperature on 120 rpm shaker. The enriched sample was further transferred into freshly prepared enrichment media each week. Serially diluted final enriched media were spread-plated on LB agar supplemented with phenol (50 mg/L) and incubated at 30°C.

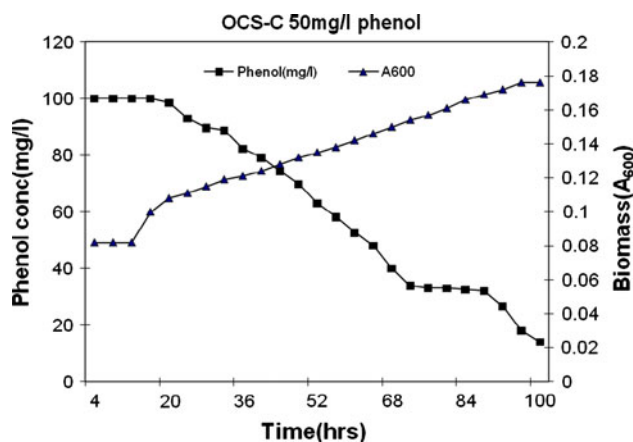
**Fig. 2** Degradation profile by OCS-A of 100 mg/L phenol**Fig. 3** Degradation profile by OCS-C of 50 mg/L phenol

The single colonies were streaked onto LB agar plates, incubated at 30°C overnight and the pure isolates were stored at 4°C until further use.

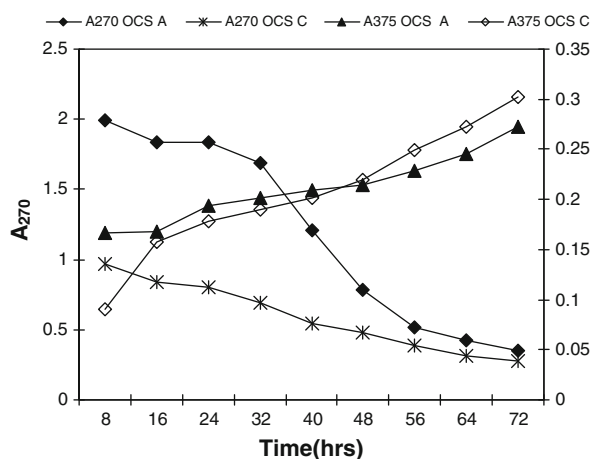
Colony morphology of the bacterium was determined by cultivating the isolates on MP and MSM medium supplemented with 50 mg/L phenol. Physiological and biochemical tests were performed including Gram's staining, Acid fast staining, Endospore staining, Capsule staining, amylase, gelatinase, citrate utilization, indole test etc. (Aneja 2001). Bergey's manual of determinative of bacteriology was used as reference to identify the isolates (Goodfellow 1994).

The isolates OCS-A was inoculated into MP medium and OCS-C were inoculated into MSM containing 10 mg/L phenol as carbon source for 48 h shaken at 150 rpm. The acclimatized isolates were further exposed to higher concentration of phenol subsequently.

The OCS-A and OCS-C bacterial isolates were grown overnight in nutrient broth and the grown bacteria were centrifuged for the 15 min at 3000 rpm. The cells were



**Fig. 4** Degradation profile by OCS-C of 100 mg/L phenol



**Fig. 5** Determination of 2-HMS intermediate with phenol degradation by OCS-A and OCS-C

resuspended in MP and MSM media, respectively having phenol (10 mg/L) as sole carbon source for degradation study. The reaction containing all components but devoid of bacterial inoculums were used as control. The concentration of phenol was increased from 10 to 100 mg/L.

The residual phenol concentration was determined by analyzing sample regularly after 4 h interval using UV visible spectrophotometer 1606 Systronics colorimetrically by 4-amino antipyrine method (Yang and Humphery 1975; APHA 1992). The cell growth (biomass) was monitored at 600 nm.

The bacterial strains OCS-A & OCS-C were spread on LB medium & incubated overnight. A 50 mg/L phenol solution was spread into the plate. The bacteria were incubated at 37°C for 25 min. The plates were eluted with a small amount of phosphate buffer (0.2 M, pH 7.0) & centrifuged at 10,000 rpm for 4°C. The supernatant was measured spectrophotometrically at 270 and 375 nm after removing the cells by centrifugation (Chen et al. 2003).

## Results and Discussion

Two bacteria capable of degrading phenol were isolated from the soil sample collected near oil industry and coded as OCS-A and OCS-C. These isolates were enriched in MP and MSM media, respectively, supplemented with phenol. These strains were characterized using morphological and biochemical properties listed in Table 1. By comparing these characteristics with those mentioned in Bergey's manual of systematic Bacteriology, the OCS-A was identified as *Citrobacter freundii* and OCS-C as *Proteus mirabilis*.

The degradation performance of selected strains was examined at different phenol concentrations. Serial exposure to increasing level of phenol concentration was used to determine the resistance of isolated strains.

The time dependant changes of phenol concentrations in the media were evaluated. Strains OCS-A and OCS-C were able to degrade phenol almost completely (about 90%) within a relatively short time of 80 h. The phenol tolerance and growth of the isolates at elevated level of phenol concentration was investigated. Cells growing at high phenol concentration (100 mg/L) showed a longer lag time compared to those growing at low concentration (50 mg/L) (Figs. 1, 2, 3 and 4).

Phenol and 2-hydroxy muconic semialdehyde (2-HMS) are starting and ending compounds of the bioconversion which show absorbance maxima at 270 and 375 nm, respectively (Quilty and Farrell 1999). OCS-C showed absorbance (0.970) at 270 nm. It was less than control (3.325) but which was decreased gradually while absorbance at 375 nm was increased progressively (Fig. 5).

The respective increase and decrease in absorption as 270 and 375 nm indicated the formation of 2-hydroxy muconic semialdehyde as a result of extradiol ring cleavage of phenol (El-Sayed et al. 2003). These results indicate that OCS-C metabolized phenol by *meta*-pathway. The same pattern was followed for OCS-A.

In conclusion, we have isolated two bacteria with capability of phenol tolerance and degrading potential. Isolates were identified on the basis of Bergey's manual of systematic Bacteriology which degraded phenol by *meta* cleavage pathway.

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