Innovative Romanian Food Biotechnology *Vol. 7, Issue of September, 2010*

Received August 26, 2010 / Accepted September 25, 2010

RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF PHENOL DEGRADING BACTERIA FROM OIL CONTAMINATED SOIL

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Abstract

Microorganisms plays a major role for saving our environments by degrading xenobiotic compounds chemicals wastes, which are toxic either in their native form or modified to be toxic. Isolation of microbial strain able to degrade chemical compounds was started usually from polluted sources, such as soil. In present study, aerobic bacteria isolated from soil contaminated with industrial xenobiotic compounds using enrichment technique containing phenol as sole source of carbon and energy was isolated in pure culture and selected for their ability to degrade phenol. The soil bacterium was identified as *Streptococcus epidermis* coded as (OCS-B). The selected microbial strain was able to degrade phenol up to 200mg/l which was also confirmed by HPLC analysis and so can be effectively used for bioremediation of phenol contaminated sites. Degradation intermediate compounds were also determined. Outcome of this study offer a useful guideline in evaluating potential phenol degraders from the environment.

Keywords: enrichment technique, bioremediation, contaminated sites

Introduction

Currently, biodegradation of aromatic compounds received a great attention from many people from industries and researchers due to their toxicity and refractory. Among all those aromatic compounds, phenol and their derivatives are known as a common constituent of soil contaminated from many industries including oil refineries, pharmaceutical, petroleum, textiles and coal refining etc. The toxicity of phenol has been widely documented and their disastrous effect toward human and environment is greatly concerned. It causes negative effects to aquatic flora and fauna (Ghadhi & Sangodkar, 1995). The maximum permissible limit of phenolic compounds in leachats for safe disposal to inland surface water is 1 mg/l. It is greatly concerned pollutant and included in list of EPA (1979). 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). By the biological degradation microorganisms and enzymes are capable to converting phenol into nontoxic intermediates of tricarboxilic acids via Ortho or Meta pathway (Powlowski and Shinglar, 1994)

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The present study aimed to evaluate the potential of a selected bacterial strain to phenol biodegradation from contaminated soil.

Materials and Methods

Isolation of bacterial strain by enrichment method

The soil sample was collected from Manas oil – Industry area, station road Pachora, Dist- Jalgaon in India. A quantity of 1gm of soil sample was suspended in 100 ml of minimal salt medium containing Na_2HPO_4 (6 g), KH_2PO_4 (3 g), NaCl (0.5 g), NH_4Cl (1 g), $CaCl_2.2H_2O$ (1 M) and $MgSO_47.H_2O$ (1 M) in 1000 ml double distilled water. 10mg/l of phenol was used as sole source of carbon and then incubated in 250ml flask at 37^0C 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/, and then incubated at 30^0C.The single colonies were streaked onto nutrient agar plates, incubated at 30^0c overnight and then the pure isolates were stored on LB agar slant supplemented with phenol as sole carbon source at 4^0c 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 etc (Nagamani *et al*, 2009). Bergey's manual of determinative of bacteriology was used as a reference to identify the isolates (MacFaddin, 2000).

Strain selection based on phenol acclimatization

The isolate coded as OCS-B (oil contaminated soil isolate B) was inoculated into MSM (mineral salt medium) medium containing 10mg/l phenol as carbon source, for 48 hrs, by shaken at 120 rpm. After 24 hrs de growth of cells was determined by turbidity measurement at 600 nm. The concentration of phenol was increased from 10mg/l to 100mg/l subsequently.

Phenol degradation studies

Bacterial isolate stain coded OCS-B was grown in the Nutrient broth by incubation at overnight, at 37^0C 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, and cultivation in submerged conditions at 37^0C, at 120 rpm for 92hrs. The reaction mixture containing all components but devoid of bacterial inoculums were used as control. Then same procedure was followed by increasing concentration of phenol from 10mg/l to 200mg/l.

The phenol concentration was determined by analyzing samples at each 4hrs interval by using UV spectrophotometer 166 Systronics. The residual amount of phenolic compounds present in the sample at different incubation period were measured by colorimetric assay 4-amino antipyrine method (Yang and Humphery, 1975; APHA, 1992)**.** The cell growth was monitored by optical density measurement at 600nm.

A HPLC analysis was carried out after 10 days of cultivation. The HPLC analysis was performed with phenonmenex C-8 column with methanol and water (70 : 30 v/v) as the mobile phase, flow rate was 1.0ml/min. The biotransformation compounds formed by phenol were monitored by the YOUNLIN INSTRUMENT MODEL NO-acme 9000.

Results and Discussion

Bacteria isolation and identification

Soil contaminated with industrial oil was chosen as the source of indigen microorganisms isolation in this study. High probability of the presence of toxic pollutants in this area was reason why site was selected (Nuhoglu and Yalcin, 2004).

Up to the one month, 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.

Table 1: *Morphological and biochemical characteristic of bacterial strain able to growth on medium with phenol as unique carbon source*

Characteristics	Bacterial strain coded OSC-B
Gram Staining	Gram positive
Shape	Coccid
Motility	Positive
Capsule staining	Capsulated
Acid Fast nature	Non Acid Fast
Endospore	Non Spore Forming
Oxygen Requirement	Aerobic
Colony	Yellow slimy
Catalase	Positive
Urease	Positive
Amylase	Positive
Gelatinase	Positive
Pectinase	Positive
H_2S production	Positive
Nitrate reduction	Negative
Indole Production	Negative
Methyl red test	Positive
Vogus Proskaur Test	Positive
Citrate Utilization	Positive
Maltose	Positive
Lactose	Positive
Sucrose	Positive
Fructose	Positive
Galactose	Negative

After treatment, three bacterial strain isolates were survived, and identified as phenol degraders. The bacterial isolate coded as OCS-B have the best potential to phenol biodegradation based on high resistance of this xenobiotic compound. The bacterial isolate was morphologically and biochemically typified and properties were listed in Table 1.

According to Bergey's manual of Determinative of Bacteriology, 90% of results showed the similarity in characteristics with *Streptococcus epidermis* (Goodfeelow *et al*, 1994).

Phenol biodegradation studies

Soils contaminated by oil are the most potential source to isolate high performance phenol degrading microorganisms. The degradation performance of selected strains was examined of different phenol concentrations. Serial exposure to increasing level of phenol concentration was used to determine the resistance of isolate strain. Acclimatization of the microorganisms overcomes the substrate inhibition problems that normally occurred in phenol biodegradation at high concentration (Lob and Tar, 2000). The experiment aimed to find the highest tolerance level of phenol concentrations found that it able to survive and degrade up to 200 mg/l phenol. The growth of bacteria and phenol concentration in media showed the inverse proportion with each other. The decrease in phenol concentration accompanied with increase in biomass (Figure 1 to 3).

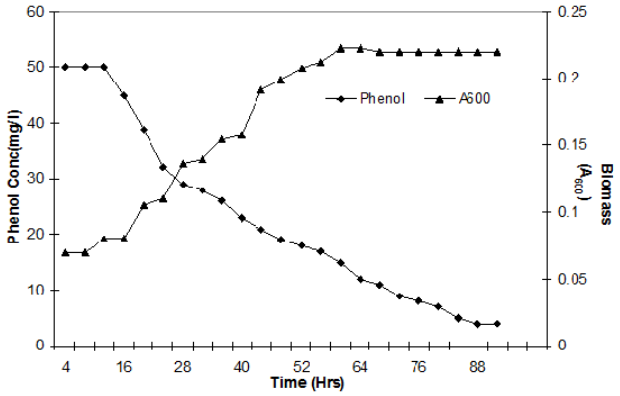

Figure 1: *Growth of selected strain Streptococcus epidermis OCS-B and phenol biodegradation at 50mg/l phenol in culture medium*

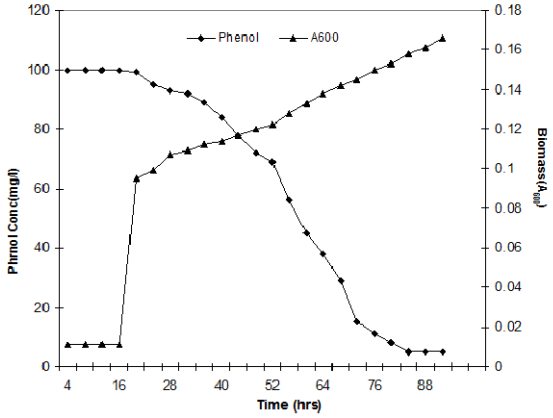

Figure 2: *Biodegradation potential of Streptococcus epidermis OCS-B strain at 100mg/l phenol in medium of growth*

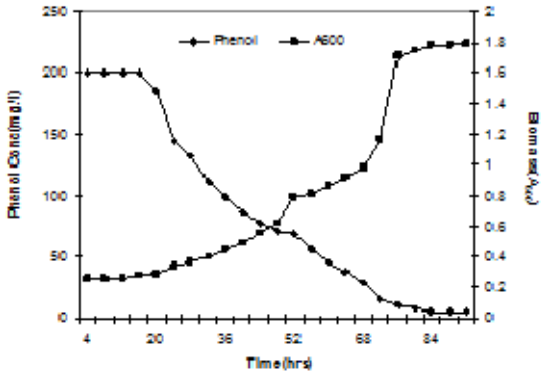

Figure 3: *Biodegradation potential of Streptococcus epidermis OCS-B strain at 200mg/l phenol in medium of growth*

In HPLC analysis of culture medium with phenol shows no peak at 270 nm which indicate that there was complete degradation of phenol compared with standard phenol, but it showed a peak on 3.400 and 3.05 RT which might indicate the formation of other intermediates compounds (Figure 4). The presence of these was confirmed spectrophotometrically by measuring absorbance at λ=375nm (Figure 5). It may be due to the presence of yellowish compound 2-hydroxymuconic semialdehydes (HMS) (Fujii *et al,* 1997). Production of hydroxymuconate semiladehyde as a result of *meta* ring cleavage of many aromatic hydrocarbons have been reported (Kaschabek *et al*., 1998; Sung *et al*., 1996; Hollender *et al.,* 1997). Hydroxymuconate semiladehyde is a precursor for down stream compounds that finally linked to the tricarboxylic acid cycle.

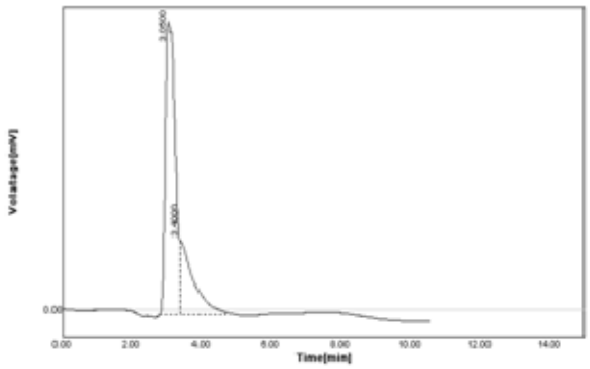

Figure 4: *Chromatogram of HPLC analysis of phenol biodegradation by Streptococcus epidermis OCS-B selected strain*

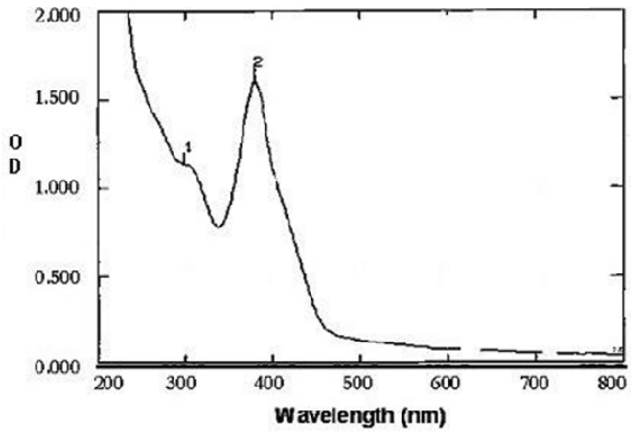

Figure 5: *Spectral scan of phenol biodegradation intermediates by Streptococcus epidermis OCS-B selected strain*

Conclusion

Till date several works are in progress to isolate new and efficient microbial strain that have ability to degrade phenol. We report here a new bacterial strain, isolate as a potential selected strain to utilize phenol as sole source of carbon and energy. The degradation ability of isolate was checked up to 200mg/l concentration. The determination of intermediates of phenol degradation was also determined. This work has provided a useful guideline in evaluating potential phenol biodegraders isolated from environment.

Acknowledgement

The authors are thankful to University grants commission (UGC), New Delhi, India for financial assistance and principal M J College to permit to carry out the study.

Financial Support

UGC Sponsored CPE (Center for potential of excellence) summer research project scheme to M.J. College, Jalgaon (MS), India

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