Isolation, characterization and optimization of catechol degrading *Pseudomonas aeruginosa* from Cashew Industrial soil

Parvathy.G, and Prabhakumari.C

*Department of Biotechnology, CEPC Laboratory and Research Institute, Mundakkal, Kollam, India.*

**Abstract**

Catechol is a reaction intermediate in the bacterial metabolism of phenol, benzoic acid, anthranilic acid and other compounds. Among the most abundant environmental pollutants; catechol and related products are of major concern because of their long term persistence and the toxicity. Industrial emissions and disposal, treatment or recycling must comply with applicable regulations to preserve environment. Therefore, the removal of catechol is vital before letting it into the environment. The present study made an attempt to find out the biodegradation of catechol by using microorganisms isolated from cashew industrial soil. Eleven morphologically different strains were isolates. Among these microbes one bacteria shows promising degradation of catechol up to 100 mg/l. Morphological and Molecular studies was done and identified as *Pseudomonas aeruginosa*. Batch studies are done by using the pure culture of *Pseudomonas aeruginosa*, catechol degradation was setup at various pH (5, 6, 7, 8, 9) and temperature (10, 20, 30, 40, 50)°C. Maximum catechol degradation was at pH 7 and temperature 30°C. From this study we can concluded that the *Pseudomonas aeruginosa* is one of the efficient catechol degraders and has wide application in the field of bioremediation.

**INTRODUCTION**

One of the worldwide problem faced by environment is pollution and its potential to influence the health of human populations is great (Fereidoun et al, 2007; Progressive Insurance, 2005). The significance of environmental factors to the health and well-being of human populations is increasingly apparent (Rosenstock 2003; World Health Organization [WHO], 2010b) in the densely settled urban-industrial Pollution.
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reaches its most serious problem.(Kromm, 1973). Over the last three decades there has been increasing global concern over the public health impacts attributed to environmental pollution (Kimani, 2007). Environmental pollutants are compounds that are toxic to living organisms; released into the ecosystem at high concentrations, usually as a consequence of human activities. Catechol and related products are of major concern because of their long term persistence and the toxicity. The International Agency for Research on Cancer (IARC) has classified catechol as a Group 2B, possible human carcinogen.

Catechol used as industrial reagent in the manufacturing of dyes rubber plastics and pharmaceuticals and cosmetics, in the production of insecticides in metal plating and coal refining. Catechol is very soluble in water and readily biodegradable. It has a low potential for bioaccumulation. Various treatment strategies are available for its removal; (M. Stanisavljevic and L. Nedic., 2004). Phenol biodegradation has been done by applying different kinds of microbial culture in two recent decades. Many aerobic bacteria have been confirmed to use aromatic compounds as the sole source of carbon and energy (Paller et al., 1995), which suggests to use catechol as nutrient to the organism and thereby converts catechol to nontoxic component. However, its removal by biological means is much cheaper, less energy consuming and above all, environment friendly.

India is largest producer, processor, exporter and second largest consumer of cashew in the world. Kerala is the main processing and exporting center of cashew. The industry provides livelihood for about 6-7 lakhs of employees and farmers, the cashew industry has national importance. (Anonymous, 2009). The soil near to cashew industry contains cashew nut shell liquid, a phenolic compound that is oozing out from the cashew nut shell during the processing of cashew nut, contains a wide variety of microbial population which has ability for degrading phenolic compounds. Biological methods for the removal of phenolic compounds are possible because some organisms have the capacity to degrade phenol utilizing it as their nutrients (Kanekar et. al, 1999, Catia et.al, 2010). The aim of this work was to isolate catechol degrading microorganisms from cashew industrial soil.

MATERIALS AND METHODS

Sampling

In this study the soil samples were collected from cashew industry near Kollam, Kerala. The samples were put into sterile bottles, then into the containers full of ice and then transferred to lab and stored in the refrigerator at temperature 4°C prior to analysis.
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Isolation of catechol degrading bacteria

For this experiment microorganisms were isolated from three different sites in cashew industry near Kollam, Kerala. Pure colonies were isolated from spread plate method using mineral salt medium (MSM) containing 1% v/v catechol. Firstly, different types of colonies were selected and taken by using sterile loop and streaked onto mineral salt medium agar plate containing 1% v/v catechol concentration. Then, the plates were incubated overnight at 30°C. The growth of pure colonies was observed after 24 hours.

Screening of catechol degrading bacteria

Eleven different bacteria were labeled and cultured on nutrient agar and incubated for 24 hours at 37°C. Then, a single colony of each bacteria was inoculated into nutrient broth and incubated at 37°C for 24 hours at 150 rpm. Then, the broths were centrifuged at 4000 rpm at 4°C for 15 minutes. Supernatants were decanted and pellets containing bacterial cells were centrifuged with 0.8 % NaCl twice to ensure removal of all broth components. The supernatant was thrown away and the pellet was centrifuged again with 10 mL of 0.8% solution NaCl.

The centrifuged pellet of eleven bacteria were inoculated into 250mL conical flask containing 100 mL mineral salt medium (Zajic and Supplison 1972) and 10 ppm catechol. The medium was adjusted to pH 7.0. Each sample was assayed in replicates. The samples were incubated at 150 rpm for 4 days, at 37°C. 1 mL of culture was centrifuged at 5000 rpm for 10 minutes and assayed the biodegradation of catechol colourimetrically by Folins Ciocalteous Method at 12 hour interval of time using UV-VIS spectrophotometer and measured at 555 nm.

Identification of catechol degrading bacteria

Identification was done on one isolated catechol degrading bacteria, were characterized and identified by their morphological characteristic based on size, shape and colony morphology on nutrient agar plate. The isolates were examined by gram staining and Biochemical tests.

16S rRNA sequencing

Genomic DNA was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel). Sequencing of 16S rRNA region using universal primers 5’CAGGCCTAACACATGCAAGTC3’, 5’GGGCWGWTGTACAAGGC3’
Catechol degradation under varying initial catechol concentrations.

10 ml of the isolate was centrifuged and the pellet was washed with 0.8% NaCl. Then the pellet was added to 100 ml of mineral salt media containing different concentration of catechol ie. 5, 10, 50, and 100mg/L and carried out in standard flask culture experiments. These flasks were kept in dark to avoid photo decomposition. 1ml of sample were removed at different time intervals and microbial growth was monitored by UV-Visible Spectrophotometer at 550nm. Then the samples were clarified by centrifugation at 5000rpm for 10 minutes and supernatants were subjected to Folins-Ciocalteus spectrophotometric method for monitoring the catechol concentration.

Effect of different pH and temperature on catechol degradation.

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RESULTS AND DISCUSSIONS

Isolation of catechol degrading bacteria

Three soil samples were collected from three different sites of Cashew Industry near Kollam. Eleven different isolates are isolated, out of which one bacteria with high potential to degrade catechol was selected for further studies.

![Figure 1](image_url). Isolate on nutrient agar.
Identification of catechol degrading bacteria

The potential isolate was characterized based on their gram reaction characteristics, morphological features and biochemical properties. The results showed that the isolate is a gram negative rod and the biochemical characterization are explained in the table.(Table:1)

Table: 1 Biochemical Characterization of the isolate

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test</th>
<th>Response of the organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gram staining</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Indole</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Methyl Red</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Voges proskauer</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Simmon citrate agar</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Oxidase</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Gelatin Liquefaction</td>
<td>+ (Rapid liquefaction)</td>
</tr>
<tr>
<td>9</td>
<td>Triple Sugar iron Agar</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Nitrate reduction</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Lactose</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Fructose</td>
<td>-</td>
</tr>
</tbody>
</table>

(+ = Positive reaction, - = Negative reaction)

16S rRNA sequencing

Genomic DNA was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel). Sequencing of 16S rRNA region using universal primers 5’CAGGCCTAACACATGCAAGTC3’, 5’GGGCGGWGTGTACAAGGC3’
Figure 2: DNA

Figure 3: PCR
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**Figure 4.** Phylogenetic analysis of strain *PSEUDOMONAS AERUGINOSA PG4* 16srRNA gene sequence with other *PSEUDOMONAS AERUGINOSA* species/strains

### Catechol degradation under varying initial catechol concentrations.

The isolate has an ability to degrade phenol up to 100 mg/l (Fig:6). 100 ppm was the initial concentration after that a rapid decrease in phenol concentration in 96 hour. This shows that the isolate has a potential ability to degrade the phenol and has a wide application in the field of bioremediation. Catia *et al* (2010) (Figure 5)
**Figure 5**: Catechol degradation under varying initial catechol concentrations.

**Figure 6**: Effect of different pH on catechol degradation.
Effect of different pH and temperature on catechol degradation.

To determine the effect of temperature and pH on phenol degradation the experiments were carried out at different temperatures such as 10°C, 20°C, 30°C, 40°C and 50°C at pH ranges from (5,6,7,8,9). The data shows that there was maximum phenol degradation takes place at room temperature of 30°C and on further increase in temperature the rate of biodegradation decreases because the catalytic activity of the enzymes is starts to decrease beyond that temperature. So the optimum temperature for the maximum enzymatic activity is 30°C and for pH, the results show that there was maximum phenolic degradation occurs maximum at neutral pH due to maximum utilization of carbon source (Figure 6&7).

At acidic or basic pH there is reduction in phenolic degradation due to the fact at that culture utilize less carbon source. Viraraghavan and Rao (2002), used the cells of Isolate .to treat the effluent of many waste water treatment plants to remove the phenol from aqueous solution. Most of the organisms, cannot tolerate the pH values below 4.0 and above 9.0 as because the acids and bases which can easily entered in to the cell which affect the metabolic pathway and denature the proteins finally leads to lethality Bandyopadhyay, et al (1998) & Annadurai et al (2000).
CONCLUSION
From the above study it was concluded that the catechol is one of the most important effluent of so many industries and it is harmful to the human system, so it has to be removed. Biodegradation is a simple, cost effective method for the removal of catechol and other effluents to protect the environment. In the present study we isolate the *Pseudomonas aeruginosa* from Cashew Industry near Kollam for the biodegradation of phenol. The Isolate degrade phenol up to 100mg/l. The catechol degradation by *Pseudomonas aeruginosa* was maximum at room temperature of 30°C and the degradation of catechol is maximum at neutral pH. Bioremediation is one of the most effective method for the removal of catechol and it has wide application for removing environmental pollutants.

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REFERENCE


