Isolation of PNP Degrading Bacillus from Soil In The Vicinity Of Coconut Husk Compost

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ABSTRACT

Industrialization increases the level of hazardous organic pollutant, and metal pollutant to the environment. Paranitrophenol (PNP) is a compound used for the synthesis of pesticides and azo dyes. In this study bacterial resistance to such a toxic pollutant was analyzed. The soil surrounding the coconut husk compost was used as a source of microorganism that will degrade PNP. The bacterium was identified as Bacillus sp using biochemical assay. The maximum activity of PNP degrading Bacillus was observed in 100mg/liter.

KEY WORDS: PNP, Coconut, Soil.

1. INTRODUCTION

Biological approach to degrade the various pollutants is growing method to clean up our environment. Debomanda Ningthoujam (2005) had reported that Brevibacteria strain was able to degrade PNP upto 300-mg/l. Microbial degradation of PNP has been reported for several bacterial species including Arthrobacter, Bacillus, Flavobacterium, Moraxella and Pseudomonas. Inhalation of PNP causes irritation to the respiratory tract, resulting in coughing and shortness of breath. Ingestion of PNP causes formation of methenoglobin, resulting in cyanosis headaches and dizziness. PNP is used as an intermediate for the synthesis of a number of organophosphate pesticides. This compound is used on a large scale in the synthesis of the aspirin. Some end products derived from PNP include dyes and pigment, fungicides and rubber chemicals. Nitro phenols are used as pH range indicators. PNP has been used as a substrate for cytochrome P450 2E1 although the major urinary metabolite is its glucoromide conjugate. PNP is both a breakdown product and a metabolite of parathion and its derivates.

2. MATERIALS AND METHODS

Soil samples were collected from around the vicinity of coir waste compost and was transferred to the laboratory and stored at –20º C. The sample was inoculated into 100 ml of minimal salt medium and incubated at 37ºC with at a speed of 200 rpm for 48 hours. Growth was observed by the turbidity in the conical flask. 1 ml of inoculum was transferred to the minimal salt medium with glucose concentration of 1 gm/L and PNP concentration of 100 mg/L and incubated for 48 hours. Coconut husk acted a carrier of phosphate solubilizing bacteria especially Bacillus and hence was used to investigate the PNP degrading activity in this study.

3. RESULTS AND DISCUSSION

The soil bacterium was grown on Nutrient Agar medium by Streak plate method. Yellow coloured colonies were observed and it was identified as gram positive rod shaped capsulated bacterium and by endospore staining green color spores were observed. The biochemical assay of confirmed the presence of Bacillus sp as shown in Table 1. The isolate was efficient in metabolizing 1.2% w/v PNP added to 100 ml of mineral salt medium. Bacillus sp could degrade PNP up to a maximum level 100 mg/l (Fig 1). The PNP degrading activity of bacillus was found to be maximum at 37ºC and pH 5-6. After 48 hrs of incubation it was observed that the PNP degrading bacteria could not utilize glucose and lactose.

Table 1. Biochemical test for identification of Bacillus sp

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biochemical tests</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catalase Test</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Methyl red Test</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Indole production Test</td>
<td>-ve</td>
</tr>
<tr>
<td>4</td>
<td>Voges-Proskauer Test</td>
<td>-ve</td>
</tr>
<tr>
<td>5</td>
<td>Glucose-lactose utilization Test</td>
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</tr>
<tr>
<td>6</td>
<td>Citrate utilization Test</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td>Urease</td>
<td>+ve</td>
</tr>
</tbody>
</table>
4. CONCLUSION

From this study it was found that the Bacillus sp isolated from the soil surrounding coir waste compost was found to degrade PNP. Thus the Bacillus sp can be used to protect our environment against pollution. Further work has to be carried out to substantiate these findings.

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