Isolation of microorganism from soil contaminated with degraded paper in Jharna village

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Pulp and paper mills are categorized as a core sector industry and are the fifth largest contributor to industrial water pollution. The pollutant, discharge from the pulp and paper industry, affect all aspects of the environment such as water, air and land. Various studies have reported detrimental effects of pulp and paper mill effluent on animals living in water bodies receiving the effluent. The effects are in form of respiratory stress, oxidative stress, liver damage and genotoxicity. This study aimed to determine the species of fungi present in the soil environment of Jharna area. Different types of fungi were isolated from soil samples contaminated with degraded papers. Identification of fungi was carried out by culturing on Potato dextrose and Sabouraud’s Dextrose agar media and microscopic method. In our study, dominant fungal flora, that is, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terrus, Penicillium sp., Trichoderma sp. and yeast were isolated. These fungi play important role in the paper and pulp industry in degradation of paper waste.

Key words: Pulp, paper, fungi, Aspergillus sp.

INTRODUCTION

The production, use and recycling of paper can have a number of adverse effects on the environment, that is, pollution. Pulp mills can emit various waste products to the air, water, and land. Discarded paper is a major component of many landfills sites, accounting for about 35% by weight of municipal solid waste before recycling (US EPA, 2005). Pulp and paper production is a major industry in India, with a total capacity of over 3 million tons per annum (CPCB, 2001). Most paper is made from cellulose pulp from tree logs. Cellulose is the most common organic crystalline polymer. Microbial degradation of lignocellulosic waste and the downstream products resulting from it is accomplished by a concerted action of several enzymes, the most prominent of which are the cellulases, which are produced by a number of microorganisms (Sukuraman et al., 2005). Cellulases are a group of hydrolytic enzymes capable of hydrolyzing cellulose to smaller sugar components like glucose units. Cellulolytic enzymes play an important role in nature’s biodegradation processes where plant lignocellulosic material is efficiently degraded by cellulolytic fungi and bacteria. In industry, these cellulolytic enzymes have found novel application in the production and processing of chemicals, food and manufactured goods such as paper, rayon and cellophane (Mandels, 1985; Kader et al., 1999). Microorganisms play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes and even protozoa. Most commonly studied cellulolytic organism include fungal species are Trichoderma, Humicola, Penicillium, Aspergillus; Bacteria-Bacilli, Pseudomonas, Cellulomonas; and Actinomycetes-Streptomycyes and Actinomucor. Trichoderma .reesei, Humicola, Penicillium
Table 1. Physiochemical properties of soil samples.

<table>
<thead>
<tr>
<th>Physiochemical property of soil</th>
<th>Readings</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.0 ±1.4</td>
</tr>
<tr>
<td>Temperature</td>
<td>31 °C</td>
</tr>
<tr>
<td>Humidity</td>
<td>36% Fair</td>
</tr>
<tr>
<td>Air pressure</td>
<td>749 mb</td>
</tr>
</tbody>
</table>

Table 2. Fungal growth in the soil environment of Jharna area using two different media.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Growth of fungi on PDA</th>
<th>Growth of fungi on SDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>+4</td>
<td>+4</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>+3</td>
<td>+2</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>+3</td>
<td>+2</td>
</tr>
<tr>
<td>Aspergillus terrus</td>
<td>+4</td>
<td>+4</td>
</tr>
<tr>
<td>Aspergillus nidulans</td>
<td>+2</td>
<td>+2</td>
</tr>
<tr>
<td>Penicillium chrysogenum</td>
<td>+4</td>
<td>+4</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>+2</td>
<td>+2</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>+3</td>
<td>+4</td>
</tr>
<tr>
<td>Yeast</td>
<td>+3</td>
<td>+3</td>
</tr>
</tbody>
</table>

Growth was scored in the following manner: +4: Excellent growth; +3: Good growth; +2: Fair growth; +1 poor growth, 0: no growth.

Materials and Methods

Study area

The study was carried out in the soil environment of Vedant Gyan Valley, Jharna village, Jaipur, Rajasthan State, India. Jharna is 48 km away from Jaipur and located on east longitude-75°27'38" and north latitude-26°49'34" and situated on altitude 450 to 500 masl.

Samples collections

Soil samples of 200 g were collected from 5 different sites in Jharna village. The samples were collected with small sterile shovels into sterile plastic containers. The soil samples were sent to the laboratory within 30 min for analysis. The pH and temperature of soil samples were determined using digital pH meter and thermometer respectively. Humidity and air pressure was determined by hygrometer and barometer respectively.

Isolation and identification of fungal isolates

Potato dextrose (PDA) and Sabouraud’s dextrose agar (SDA) media were employed for the isolation of fungi by spread plate method using serial dilution technique. All the plates were incubated at 30°C for 7 days. Fungal isolates were identified by cultural and microscopic features.

RESULTS

In our results, the soil of Jharna village was slightly acidic, the humidity and temperature was 36% and 31 °C respectively. The physiochemical properties of soil play an important role in the growth of microorganism. Tables 1 and 2 show physiochemical properties of soil and isolated fungi. In our findings, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terrus, Penicillium sp., Trichoderma sp. and yeast were found (Figure 1). A. niger and Penicillium sp. were dominating species, they show vigorous growth and are found in large numbers. A. niger has black colour colony with conidial production on potato dextrose media. A. niger is biseriate, conidiophores are long, smooth and hyaline, becoming darker at apex and terminating globose vesicle, metulae and phialides cover the entire...
Figure 1. The growth of fungi on potato dextrose agar (a) A. terrus; (b) A. nidulans; (c) P. chrysogenum; (d) Mucor sp. (e) A. fumigates; (f) A. niger; (g) Trichoderma sp.; (f) A. flavus.
vesicle, conidia are brown to black very rough and globose and measures 4 to 5 µm in diameter. A. terrus have a cinnamon colour colony on potato dextrose agar media, hyphae are septate and hyaline, and conidial heads are biseriate; conidiophore are smooth walled and hyaline, terminating mostly globose vesicle, conidia are small globose and smooth and measure 2 to 6 µm in diameter. A. flavus have a green color colony on potato dextrose agar media, hyphae are septate and hyaline, conidiophores are coarsely roughened, globose vesicle, metulae covering the entire vesicle, conidia are smooth walled and 3 to 6 µm in diameter. A. fumigates have gray-green colony in potato dextrose agar. Hyphae are septate and hyaline, conidiophores are smooth walled, uniseriate with closely compact phialides occurring only on the upper portion of the columnar vesicle. Conidia are smooth to finely roughed and 2 to 3 µm in diameter. Mucor show white cottony growth on potato dextrose agar media, nonseptate hyphae, having sporangiphore, sporangia and spores. Trichoderma colony is transparent at first on pda, after seven days of incubation at 25 to 28°C, green colour tufts conidia form. Conidiophores are highly branched.

DISCUSSION

Fungi have unique bioremediation properties. Many of the remediation technologies currently being used involve not only physical and chemical treatment, but also biological processes. A. flavus, A. niger and Rhizopus sp. were also isolated from paper mill effluents (Bajwa et al., 2010). Raju et al. (2007) also isolated A. flavus and Trichoderma sp. from the polluted soil samples of paper mills of South Indian paper mills. Jahangeer et al. (2005) also isolated Aspergillus, Alternaria, Trichoderma, Penicillium and Rhizopus sp. from the soil samples. The results would also be useful to environmental agencies that are concerned with the management of solid wastes. A number of species of bacteria and fungi were isolated and partially characterized from enrichment cultures containing the fungicide thiram (Sahin and Tamer, 2000). Our findings were also similar with Luis Henerique et al. (2010); they isolated A. niger, A. flavus, and Mucor from the soil samples. Fusarium spp., Penicillium spp., Trichoderma spp., Paecilomyces spp., Epicoccum sp. Dihieterosphora spp. and Verticillium spp. were capable of hydrolyzing EPTC from a soil previously exposed to the herbicide EPTC (Lee, 1984). Aspergillus sp., Fusarium sp., Mortierella sp. and Penicillium sp. were able to defluorinate activity 1080 from soils previously exposed to Namonofluoracetate (1080) (Wong et al., 1992). Mishra and Dadhich (2010) also isolated fungi A. niger, Penicillium and Trichoderma from soil samples of rajasthan having amylase and xylanase activity. Environmental agencies can take advantage of the result of this investigation and then invest in further studies which could lead to the isolation and characterization of specific high-yielding fungal strains found in the local soil environments. This will go a long way in reducing the cost of production in such industries as well as the cost of management of solid waste by environmental agencies.

Conclusion

A. niger, A. fumigatus, A. flavus, Trichoderma sp., Rhizopus sp. and Penicillium sp. were isolated from the soil contaminated with degraded paper. This investigation may lead to the development of strains of soil fungi that would be used locally for the biodegradation of cellulose materials. These organisms are so recommended for management of solid wastes containing cellulose.

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REFERENCES

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