Molecular Identification of Fungal Strains isolated from Textile Effluent and Contaminated Soil using 16s rRNA Sequencing
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Abstract
A study was conducted on isolation and identification of fungi in textile effluent and its contaminated soil. A total of four fungal strains were successfully isolated: three from textile industry effluent contaminated soil and one from textile industry effluent. All the four isolates were identified based on 16s rRNA gene sequencing. They were identified as Aspergillus flavus, Penicillium hetheringtonii, Aspergillus aculeatus and Aspergillus pseudonomiae.

The sequences were submitted to GenBank under accession number MZ544387, MZ574434, MZ569631 and MZ569632 respectively.

Keywords: Textile industry effluent, Fungi, NCBI, GenBank, sequence, BLAST, Accession number.

Introduction
Textile industries contribute nearly 14% of the total industrial production in India and discharge large volume of effluent after dyeing process. The effluent contains different types of dyes based on their chemical nature such as azo, diazo, cationic, basic, anthroquinone base and metal complex dyes\(^6\). Synthetic dyes (e.g. azo, xanthenes and anthroquinone) are highly toxic or mutagenic for organisms and also affect natural resources\(^4\). Textile industry effluent consists of a mixture of organic compounds of complex structure which results in increasing pollution when released into the environment\(^15\).

The discharge of such untreated textile effluent into the water resources such as rivers and lakes alters pH, increases chemical oxygen demand (COD) and gives intense coloration\(^9,12\). Textile effluents are often contaminated with non-biodegradable organic materials too. To minimize the adverse environmental hazards and human health effects, treatment of textile effluent becomes necessary before it is discharged into water bodies\(^16\).

Microorganisms are ubiquitous in nature and diverse microbial communities thrive in natural and extreme stress environments including soil, water, the human gut, hydrothermal vents, acid mine runoff and oil reservoirs. Microbial populations exhibit potential for the remediation of any contaminated environment because of genetic diversity and functionality\(^13\).

The study of microbial population existing in contaminated environments provides a significant knowledge of specific microbial characteristics that improve degradation rates. Soil is the most dynamic environment for the enormous microbial population\(^11\).

Fungi represent the second largest group of eukaryotic organisms on earth. The kingdom fungus has enormous species diversity with varied morphologies, ecologies and nutritional modes; many are of industrial importance. The fungal kingdom is estimated to comprise between 2.2 to 3.5 million species with only about 7% named and classified\(^3,14\). Fungi are considered as diverse group of eukaryotic organisms and have very important role in ecosystem\(^6\).

The aim of the present study was to isolate fungi from effluent and effluent contaminated soil. After isolation, identification and molecular characterization of the fungal strains were used for optimizing various parameters in textile effluent.

Material and Methods
Effluent collection: Effluent was collected in dry, sterile, polypropylene bottles which were kept in ice during transportation. Sample was stored in the refrigerator (4\(^{\circ}\)C) till the isolation of fungi.

Soil sample collection and preservation: Soil sampling for fungal isolation was carried out a textile mill in Bangalore. Sample was collected from polluted soil. 20-50g of soil samples from the depth of 15cm from dumping areas contaminated with effluent of textile industry were collected in sterile polythene bags. The collected soil sample was transported to the laboratory in icebox and stored in refrigerator in 4\(^{\circ}\)C for further work within 12 hours.

Analysis of physico-chemical parameters: Textile effluent was characterized in triplicate for pH, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), nitrate, sulphate, heavy metals like cadmium and lead by standard methods\(^1\).

Isolation of fungal isolate from effluent sample and soil sample: Fungi from effluent was determined by serial dilution of 10\(^{-1}\) to 10\(^{-10}\) and plating in potato dextrose agar (PDA) media. Soil sample (1g) was suspended in 10ml of sterile distilled water and further dilutions were made. To isolate fungal strains, soil dilutions of 10\(^{-1}\) to 10\(^{-10}\) were prepared. Subsequently, for isolation, 10\(^{-8}\) and 10\(^{-9}\) were...
used to avoid over-crowding of fungal colonies in soil as well as for effluent. Soil suspension of 100ul of each concentration was added to petriplates containing 30ppm streptomycin and 20ml of sterile potato dextrose agar (PDA) medium. The culture plates were incubated in laboratory conditions up to 7 days. Each developed colony was subcultured to obtain pure cultures.

**Identification of the fungal isolate:** Identification of isolated fungal colonies was carried out by the method of Anjea based on colony characteristic on PDA (colony morphology, colour, appearance) and microscopic characteristics (septation of mycelium, shape and texture of reproductive structure i.e. conidia). ITS sequence fragment results were analyzed using the Basic Local Alignment Search Tool (BLAST) in GenBank to verify identity on the National Center for Biotechnology Information (NCBI, https://blast.ncbi.nlm.nih.gov/) database and MEGA version 5. BLAST search is usually employed using nucleotide collection (BLASTn). Phylogenetic trees were generated.

**Results and Discussion**
Based on colonial morphology, appearance and color of fungal mycelia on culture plate and microscopic characteristics viz. mycelia septation, size, shape, diameter, texture and septation of reproductive structure conidia were observed (Table 1 and figure 1).

In the present study, four fungal strains were isolated from the textile industry effluent contaminated soil and effluent. The internal transcribed spacer (ITS) regions are highly preserved in most species having intraspecific resemblances greater than 100% but show variation between species which make it suitable for application in classification. ITS1 (fungal specific primer) and ITS4 (fungal general primer) are the first PCR primer sets usually employed to copy the fungal ITS regions. The ITS rRNA analysis using ITS1 and ITS4 primers confirmed the organisms to be 100% identical. The sequences derived from PCR products were subjected to a homology analysis and percent identities of 98-100% were obtained when the 18S partial gene, ITS1 region, ribosomal 5.8S gene, ITS4 region and ribosomal 28S partial gene were compared.

**Database Submission:** Based on the genetic and phylogenetic analysis, the new strains were identified as Aspergillus flavus, Penicillium hetheringtonii, Aspergillus aculeatus and Aspergillus pseudonomiae. The 16s rRNA sequences of four of these fungal isolates were deposited in GenBank. Accession numbers are listed in table 2.

The nucleotide sequences derived from the textile effluent and textile industry effluent contaminated soil fungi reported in this work were submitted to GenBank accession numbers MZ544387 (Aspergillus flavus isolate KUESCHK-1), MZ574434 (Penicillium hetheringtonii isolate KUESCHK-2), MZ569631 (Aspergillus aculeatus isolate KUESCHK-3) and MZ569632 (Aspergillus pseudonomiae isolate KUESCHK-4). Phylogenetic tree of four isolated fungal strains is presented in figure 2.

**Characterization of textile effluent:** Observed values of effluent discharged into the environment were compared with that of Central Pollution Control Board (CPCB) (Table 3).

**Table 1**  
Macroscopy and microscopy characteristics of isolated fungal strains

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Macroscopy</th>
<th>Microscopy</th>
<th>organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ544387</td>
<td>The upper surface of colonies was olive green with white edge, granular surface and white coloration on the reverse side</td>
<td>Non-septate</td>
<td>Aspergillus flavus</td>
</tr>
<tr>
<td>MZ574434</td>
<td>The upper surface of the colonies was blue-green with white edge, smooth surface and yellowish to white on reverse side</td>
<td>Non-septate</td>
<td>Penicillium hetheringtonii</td>
</tr>
<tr>
<td>MZ569631</td>
<td>The colonies were widely spread, black, spongy surface densely packed and brown on reverse side</td>
<td>Non-septate</td>
<td>Aspergillus aculeatus</td>
</tr>
<tr>
<td>MZ569632</td>
<td>The colonies were widely spread, green with black surface and light brown on reverse side</td>
<td>Non-septate</td>
<td>Aspergillus pseudonomiae</td>
</tr>
</tbody>
</table>

**Table 2**  
The NCBI database BLASTn result

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Description</th>
<th>Maximum Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ544387</td>
<td>Aspergillus flavus</td>
<td>100%</td>
</tr>
<tr>
<td>MZ574434</td>
<td>Penicillium hetheringtonii</td>
<td>100%</td>
</tr>
<tr>
<td>MZ569631</td>
<td>Aspergillus aculeatus</td>
<td>100%</td>
</tr>
<tr>
<td>MZ569632</td>
<td>Aspergillus pseudonomiae</td>
<td>100%</td>
</tr>
</tbody>
</table>
A. Surface and reverse side image of *Aspergillus flavus*

B. Surface and reverse side image of *Penicillium hetheringtonii*

C. Surface and reverse side image of *Aspergillus aculeatus*

D. Surface and reverse side image of *Aspergillus pseudonomia*

Figure 1: Macroscopy images of isolated 4 fungal strains. The photos in the figure are the original collection.
The values observed were critically higher as compared to standard values. The results are in correlation with those observed in other studies by Mondal et al\(^\text{10}\), Karthikeyan and Anbusaravan\(^\text{7}\) and Hassan et al\(^\text{5}\).

**Conclusion**

In the present study, isolation of fungi from textile industry effluent as well as textile industrial effluent contaminated soil was done. Four fungi were isolated viz. *Aspergillus flavus*, *Penicillium hetheringtonii*, *Aspergillus aculeatus* and *Aspergillus pseudomoniae* and the nucleotide sequence of four fungal strains was compared with sequences available in the NCBI database and deposited to GenBank with accession number MZ544387, MZ574434, MZ569631 and MZ569632 respectively.

The availability of fungal strains in textile effluent contaminated soil and effluent was key note for they have an ability to grow in polluted environment and survive in extreme conditions. Hence, fungi could play a very important role in bioremediation of textile wastewater.
Table 3
Qualitative parameters of textile effluent

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observed values</th>
<th>CPCB Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>9.5 ± 0.057</td>
<td>6.0-8.5</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>47 ± 1.15</td>
<td>Not exceed 40°C</td>
</tr>
<tr>
<td>Total Dissolved Solid (mgL⁻¹)</td>
<td>2689 ± 3.60</td>
<td>2100</td>
</tr>
<tr>
<td>Total Suspended Solid (mgL⁻¹)</td>
<td>377.4 ± 2.27</td>
<td>100</td>
</tr>
<tr>
<td>Biological Oxygen Demand (mgL⁻¹)</td>
<td>694 ± 4.27</td>
<td>30</td>
</tr>
<tr>
<td>Chemical Oxygen Demand (mgL⁻¹)</td>
<td>2662.6 ± 2.82</td>
<td>250</td>
</tr>
<tr>
<td>Sulphate (mgL⁻¹)</td>
<td>1454.6 ± 2.25</td>
<td>1000</td>
</tr>
<tr>
<td>Nitrate (mgL⁻¹)</td>
<td>12.3 ± 0.57</td>
<td>10</td>
</tr>
<tr>
<td>Cadmium (mgL⁻¹)</td>
<td>0.51 ± 0.03</td>
<td>0.2</td>
</tr>
<tr>
<td>Lead (mgL⁻¹)</td>
<td>0.26 ± 0.02</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*= no units for pH

Acknowledgement
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References