INTRODUCTION

Money, whether in the form of coins or paper notes, is perhaps the most widely handled article by people everyday and throughout the world. It is used in every type of commerce. During the course of transit, the currency notes and coins pass on from one hand to another, and hence many kinds of microorganisms contaminate them, which include both the pathogenic and non-pathogenic microbes. Contamination of paper notes and coins could be due to poor handling practices during production, usage, various religious ceremonies and during storage (Awodi et al., 2001). Contamination of paper currency with microbes is due to the fact that paper money provides large surface area as a breeding ground for microbes and then afterwards they can persist on it for longer periods. Moreover, fungal species, which are known to be distributed in every environment, thrive well on money, usually when the money is kept in a damp environment or circulated among persons living in unhygienic conditions (Prescott et al., 2002; Janardan et al., 2009).

The fungal organisms are known to produce an array of enzymes, including cellulases, hemicellulases, pectinases, chitinas, amylases, proteases, phytases and mannases. The paper currency notes being high in cellulose content, act as a favourable substrate for colonisation by fungal organisms, which degrade them by virtue of their cellulolytic potential. These cellulolytic enzymes are a group of extracellular hydrolytic enzymes responsible for cellulolytic and xylanolytic activities. This leads to deterioration of the paper currency notes, especially the lower denomination notes as observed in the present investigation. Fungal genera showing cellulolytic potential find various industrial applications, for example, in the textile industry (Gusakov et al., 2000; Belghith et al., 2001), in detergents (Maurer et al., 1997; Kottwitz et al., 2005), pulp and paper industry (Buchert et al., 1996), improving digestibility of animal feeds (Lewis et al., 1996), and in food industry (Galante et al., 1998). Cellulolytic enzymes account for a significant share of the world market. However, very little information is available about the cellulolytic fungi associated with lower denomination currency notes in India. The present work concentrates on the isolation of cellulase active fungal species from soiled and mint currency note samples circulating in Jammu city (India).

MATERIALS AND METHODS

Mint and soiled currency notes of lower denomination (rupees five, ten, twenty and fifty) were collected aseptically from various sources and brought to the laboratory in pre-sterilized polythene bags for isolating the mycobial diversity associated with them and assessing their cellulolytic potential. Isolation of fungal species was done by following dilution pour plate method and using modified Sabouraud Dextrose Agar medium (SDA). Five replicates were prepared and incubated for 7 days at 28±2°C till proper growth of the fungal colonies was obtained. The recovered fungal species were identified by studying their macro- and micromorphological characters. Relevant literature and various keys used for the identification of recovered fungal species were those given by Brown and Smith (1957), Ames (1961), Raper and Fennel (1965), Tandon (1968), Rifai (1969), Booth (1971), Ellis (1971), Pitt (1979) and Onions et al. (1981).

Preliminary assay of cellulolytic activity

Cellulolytic activity of the recovered fungal isolates was determined by following the method of Pointing (1999). This method gives a positive or negative indication of enzyme production by a particular fungal species and is useful in screening...
a large number of isolates. In this method, the test fungi were first
grown on cellulose basal medium (CBM) for 5-6 days. Then agar
disks were cut from the margin of the test colony and put over
CBM plated petriplate supplemented with carboxymethylcellulose
(CMC). The petriplates were incubated in darkness for
2-5 days till the colony attained a diameter of approximately
30mm. The petriplates were then flooded with 2% w/v aqueous
Congo red (C122120) and left for 15 minutes. The stain was then
poured off and agar surface was rinsed with distilled water.
The petriplates were again flooded with 1M NaCl to destain for about
15 minutes. Finally, NaCl solution was also poured off. The
Carboxymethyl cellulose degradation around the colony appeared
as yellow opaque area against red colour for undergraded
carboxymethyl cellulose. Congo red stain binds with β-1-4 linked
glycosidic bonds. Fungal strains producing cellulase will
hydrolyse all cellulose around their colonies in CBM-CMC plate
so that Congo red does not bind among these colonies and a clear
yellow opaque zone appears. The diameter of yellow opaque area
gives a direct qualitative estimate of the efficiency of cellulolytic
activity of the test fungus.

Quantitative estimation of cellulase
Fungal species producing large opaque zones were selected for
the quantitative estimation of amount of cellulase produced by
them. First of all, production medium (CBM without agar) was
prepared and about 100ml of production medium was put in each
250ml conical flasks. Then discs of the selected fungal cultures
were cut and inoculated in the production medium. The inoculated
flasks were incubated for 5-6 days at 28ºC and then centrifuged at
10,000g for 10 minutes. The supernatant, which was enzyme
equivalent was taken and was assayed by dinitrosalicylic acid
(DNSA) method as given by Miller et al., (1960). Cellulase
activity was determined by mixing 0.5ml of 1.0% (w/v) CMC
(prepared in 0.5ml of 10mM phosphate buffer pH-7.0) with 0.5ml
of crude extracellular enzyme source and incubating at 50ºC for
15 minutes (Casimir et al., 1996). The reaction was stopped by
addition of 1.5ml of 3,5-dinitrosalicylic acid (DNSA) and the
contents were boiled for 10 minutes. The addition of DNSA (3.5-
dinitrosalicylic acid) was a redox reaction in which DNSA reacts
with reducing sugars at 90ºC in alkaline condition. Reducing
sugars get converted into corresponding acids of sugars and 3,5-
dinitrosalicylic acid gets converted into 3-amino,5-nitro-salicylic
acid. 3,5-dinitrosalicylic acid has yellow colour, which changes to
orange red on reduction into 3-amino, 5-nitro salicylic acid (Fig. 1).
The colour developed was read in a spectrophotometer (Analytik jena, Specord 200) at 575nm. The amount of reducing
sugar liberated was quantified using glucose as standard (Fig. 2).
One unit of cellulase was defined as the amount of enzyme that
liberates 1µmol of glucose equivalents per assay condition
(Mandels et al., 1981) and is calculated as:

\[
1 \text{ Enzyme Unit} = \mu \text{mol/ml/min}
\]

RESULTS

Data presented in table 1 shows that among the recovered fungal
species, 27 were positive for cellulase production, whereas other
10 fungal species (Rhizopus oryzae, Mucor hiemalis, Syncephalastrum racemosum, Chaetomium globosum, Eurotium
chevalieri, Aspergillus ochraceus, Aspergillus terricola var.
americana, Aspergillus versicolor, Paecilomyces lilacinus and
Penicillium brevicaempactum) did not show cellulolytic activity.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Cellulase production (µmol/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata</td>
<td>0.28</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>0.28</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>0.31</td>
</tr>
<tr>
<td>Aspergillus japonicus</td>
<td>0.36</td>
</tr>
<tr>
<td>Aspergillus nidulans</td>
<td>0.36</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>0.44</td>
</tr>
<tr>
<td>Aspergillus ochraceus</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus parasiticus</td>
<td>0.32</td>
</tr>
<tr>
<td>Aspergillus sydowii</td>
<td>0.27</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>0.26</td>
</tr>
</tbody>
</table>
| Aspergillus terricola var.
  americana                     | -                                |
| Aspergillus versicolor          | -                                |
| Chaetomium globosum             | 0.73                             |
| Chaetomium indicum              | -                                |
| Cladosporium cladosporoides     | 0.14                             |
| Curvularia lunata               | 0.26                             |
| Curvularia pallescens           | 0.24                             |
| Emeritella nidulans var.
  echinulata                     | 0.28                             |
| Eurotium chevalieri             | -                                |
| Fusarium oxysporum              | 0.49                             |
| Fusarium palid Rosseum          | 0.36                             |
| Fusarium solani                 | 0.36                             |
| Fusarium verticilloides         | 0.38                             |
| Macor hiemalis                  | -                                |
| Paecilomyces fassporus           | 0.32                             |
| Paecilomyces lilacinus          | -                                |
| Penicillium brevicaempactum     | -                                |
| Penicillium chrysogenum          | 0.52                             |
| Penicillium citrinum            | 0.22                             |
| Penicillium expansum            | 0.32                             |
| Penicillium griseofulvum        | 0.34                             |
| Penicillium oxalicum            | 0.38                             |
| Penicillium waksmanii           | 0.26                             |
| Rhizopus oryzae                 | -                                |
| Syncephalastrum racemosum       | -                                |
| Trichoderma koningii            | 0.69                             |

(*) cellulase production not detected

Among the 27 fungal species that were positive for cellulase
production, 6 species viz., Chaetomium globosum, Trichoderma
koningii, Penicillium chrysogenum, P. griseofulvum, Fusarium
oxysporum and F. verticilloides produced large opaque zones,
whereas Aspergillus niger, A. japonicus, A. nidulans, Penicillium
oxalicum, Fusarium solani and F. palid Rosseum produced
comparatively smaller zones (Fig. 3 and Fig. 4).

![Fig. 1 Redox reaction involving DNSA and reducing sugar](image-url)
In the present investigation, 27 fungal species that produced prominent opaque zones were selected for quantitative estimation of cellulase. This was done by dinitrosalicylic acid (DNSA) method. It was found that the cellulolytic activity of the studied fungal species ranged between 0.14 to 0.73 µmol/ml/min (Table 1).

Among the 27 fungal species studied for quantitative estimation of cellulase activity, Chaetomium globosum showed the maximum cellulolytic activity (0.73 µmol/ml/min), followed in decreasing order by Trichoderma koningii (0.69 µmol/ml/min), Penicillium griseofulvum (0.54 µmol/ml/min), P. chrysogenum (0.52 µmol/ml/min), Fusarium oxysporum (0.49 µmol/ml/min) and Aspergillus niger (cellulolytic activity 0.44 µmol/ml/min). Cellulolytic activity of other fungal species ranged between 0.14 – 0.38 µmol/ml/min, whereas Cladosporium cladosporioides showed the lowest cellulolytic activity (0.14 µmol/ml/min).

DISCUSSION

In nature, hydrolysis of cellulose occurs as a result of the action of a cellulase complex produced by cellulolytic microorganisms. This complex includes endoglucanases (endo-1,4-β-glucanases), cellobiohydrolases (exo-1,4-β-glucanases) and β-glucosidases. Endoglucanases can hydrolyze internal bonds and release new terminal ends, cellobiohydrolases can act on the existing or endoglucanase-generated chain ends and β-glucosidases can catalyze the hydrolysis of terminal non-reducing residues in beta-D-glucosides with release of glucose. In humid conditions, cellulolytic fungi frequently colonize currency notes and produce extracellular cellulolytic enzymes with endoglucanase and exoglucanase activity, hydrolysing cellulose to monosaccharides.

There is very little information about the association of cellulolytic fungi with the Indian currency notes. Recently, Abirami et al., (2012) reported that most of the fungi associated with Indian currency notes showed cellulolytic activity and these included Mucor sp, Rhizopus nigricans, Helminthosporium sp., Verticillium sp., Aspergillus flavus, A. fumigatus, A. terreus, A. ochraceus, A. nidulans, A. sulphureus, A. luchuensis, A. oryzae, A. cleavatus, A. ustus, Penicillium citrinum, P. frequentans, P. funiculosum, Fusarium oxysporum, F. semitectum, F. moniliforme, Curvularia lunata, C. pallescens, Trichoderma longibrachiatum and Candida albicans. Incidentally, during the present investigation, many of these fungal species were recovered from the lower denomination notes that were in circulation in Jammu city and they were also detected to be

![Fig. 2 Standard graph of glucose (concentration range of 0.4-5.2 µm)](image)

![Fig. 3 Cellulolytic active fungal species producing large opaque zones](image)

![Fig. 4 Cellulolytic active fungal species producing small zones](image)
cellulolytic. Earlier, cellulolytic activity of Chaetomium globosum has been well documented by Eyini et al., (2002), Kolet (2010) and Ravindran et al., (2011). Similarly, cellulolytic potential of Trichoderma koningii has been reported by Halliwell and Vincent (1981) who purified 1,4-β glucanase component from it. Later, Liu and Yang (2007) also reported cellulose production by AS3.42627 strain of Trichoderma koningii in solid state fermentation by using lignocellulosic waste from the vinegar industry.

Similarly, Paecilomyces fusisporus, which is being reported for the first time from the currency notes was found to be cellulolytic (cellulolytic activity = 0.32 µmol/ml/min) and has earlier also been reported by Kapoor et al., (1978) and Kainsa et al., (1979) to be cellulolytic. During the present investigation, Aspergillus terecula var. americana, A. versicolor, A. ochraceous, Rhizopus oryzae, Mucor hiemalis, Syncphalastrum racemosum, Paecilomyces lilacinus, Penicillium brevicompactum, Chaetomium indicum and Eurotium chevalieri were recovered commonly from the currency notes but were detected to be negative for cellulase activity. However, some isolates of these fungi have been observed by other researchers to be positive for cellulolytic activity. For example, Agarwal and Chauhan (1977) reported soil isolate of Chaetomium indicum to be positive for cellulose production. Gopinath et al.(2005) reported cellulolytic potential of Syncphalastrum racemosum isolated from edible oil mill wastes. Takii et al., (2005) reported β-glucosidase activity from MIBA348 strain of Rhizopus oryzae. Karmakar and Ray (2011) also reported endoglucanase activity of Rhizopus oryzae and used it for the bioconversion of agro wastes. Begum and Alimon (2011) screened some species of Aspergillus recovered from different cellulose waste substrates for cellulase production and reported high cellulolytic potential of Aspergillus oryzae, A. ochraceous, A. flavus and A. cervinus. Recently, Sharma and Sumbali (2013) found Aspergillus fumigatus and Penicillium oxalicum recovered from ant-hill soils of Jammu city to be negative for cellulase activity, whereas in the present study their isolates from currency notes were found to be positive. This indicates that different isolates of the same fungal species may also have varied enzymatic potential. It can be inferred that a large number of fungal species are associated with the paper currency notes and these may bring about gradual deterioration of the notes by causing discoloration, foxing, yellowing and sometimes accompanied by faint to musty odour. Among the recovered fungal species, Chaetomium globosum, Trichoderma koningii, Penicillium chrysogenum, P. griseofulvum, Fusarium oxysporum and Aniger were found to be highly cellulolytic. These fungal species can be exploited as important sources of cellulase enzyme, which has multifarious applications in various industries. However, more basic research needs to be carried on these fungal species for determining their active strains and characterizing those parameters, which are required for their maximum activity.

References


