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RESEARCH ARTICLE

STUDIES ON ENZYMATIC POTENTIAL FUNGI ISOLATED FROM MUNICIPAL SOLID WASTE IN JABALPUR

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ABSTRACT

Solid wastes are dumped in open area and it's a major sources for pollution. Many organisms are present in municipal solid waste like fungi, bacteria, actinomycites and mites. Ten fungi *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus* sp., *Mucor* sp., *Alternaria alternata*, *Aspergillus glaucus*, *Sarcinella* sp., *Aspergillus ustus*, *Cladosporium* sp. and Unidentified sp. (1). were isolated from Civil line and Ranital dumping region of Jabalpur city. *A. niger* and *A. flavus* were more dominant in solid waste. Out of these 10 species 6 showed secretion of high amount of enzyme such as *A. flavus*, *Aspergillus* sp., *Mucor* sp., *A. glaucus*, *A. ustus*, Unidentified sp. (1).

INTRODUCTION

Solid Waste (SW) often called the third pollution after air and water pollution and this material is raised from various human activities and is normally discarded as useless or unwanted waste. In developing countries like India generation of municipal solid waste (MSW) is extremely high which become a problem for management by the municipalities and municipal corporation. The MSW means all waste material discarded for disposal by house hold, hotels, motels, Commercial, institutional industrial sources (Gautam *et al*., 2010a). MSW is suitable for composting because of the presence of high percentages of organic matter (Gautam *et al*., 2012).

The multiple uses of aerobic composting, anaerobic digestion and vermicomposting have drifted the attention of scientists to herbal technology, which employs microorganisms for waste biodegradation studies. The problem of MSW has been shooting very effectively and converted into opportunities to generate energy, enzyme or organic manure (Reddy *et al*., 2010). Fungi are excellent decomposers because they can break down and utilize a wide variety of complex compound (Chinedu *et al*., 2010). The activities of decomposition were measured through the secreted amount of enzyme by fungi. Fungi release different enzymes like Amylase, protease and Cellulase enzyme etc. Thus, it is essential to examine the relationship between fungi and these enzymatic activities. The present investigation was carried out to survey the fungal flora inhabiting different decomposing waste habitats and to test the enzyme production by them.

MATERIAL AND METHOD

Survey of sampling site

Jabalpur is located at 23°10′N 79°57′E23.17°N 79.95°E. The central point of India is located in Jabalpur district. It has an average elevation of 411 meters (1348 feet). MSW was collected from different place of Jabalpur city.

Sample Collection

Sample was collected from Civil line near kaushalya apartment, Opposite of Polices station and Ranital dumping area in Jabalpur city. Before sample collection wear the sterilized hand gloves and mask on the face. Then organic waste were collected in sterilized zip polythene bag with the help of sterilized spatula and mark the Date, Time, Temperature, Place, pH on the polythene bag. Care was taken

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to handle the samples under the aseptic condition and stored in refrigerator at 4ºC for the further investigation.

Isolation of fungi

The fungal species was isolated from different sample by serial dilution method from the appropriate plate in three replication. After 4 to 7 days at 28±2ºC incubation, single colonies were isolated and maintained on PDA slants at 4ºC for further investigation (Waksman, 1922; Warcup, 1950; Aneja, 2007).

Identification of fungi using lactophenol and cotton blue

In clean slide drop of lactophenol was place. Transfer a small part of the fungus preferably with spores and spores bearing structure with cool needle. The slide were carefully prepared and observed under low and high magnification power of microscope (Nagmani *et al*., 2006; Verma *et al*., 2008 after Purvis *et al*., 1994).

Enzymatic Screening of Isolated fungi

Qualitative estimation of hydrolytic enzyme by plate assay method. All 10 fungal isolates were screened for cellulase, amylase and protease activity.

Amylase activity

Prepared the starch agar medium (Starch 20gm; Peptone 5gm; Beef extract 3gm;Agar 15gm; Distilled water 1000ml) and pour into the sterile Petri dishes. Allow it to solidify. Use sterile loop, make a single streak inoculation of each organism into the center of its appropriately labeled plate. Then incubated plate for 72–96 hours at 25ºC in an inverted position. After incubation flood the surface of the plates with iodine solution with a dropper for 30 seconds. Pour off the excess iodine solution (Iodine 1gm; Potassium iodide 2gm; Distilled water 300ml). The starch hydrolysis around the line of growth of each organism (Ross, 1976; Aneja, 2007).

Protease Activity

Prepared the gelatin agar medium (Dextrose 5gm; KNO_3 3.5gm; KH_2PO_4 1.5gm; $MgSO_4.7H_2O$ 0.75gm; Agar 20gm; Casien 10gm; Distilled water 1000ml), pour into sterile Petri dishes and allow to solidify. After inoculation plate incubated for 72- 96 hours at 25ºC in an inverted position. Flood the surface of the plates with Mercuric chloride solution (1% $HgCl_2$ 10gm; Distilled water 1000ml) and allow the plates to stand for 5 to 10 minutes. The color change of the medium and zone was found (Aneja, 2007).

Cellulase activity

1% Carboxymethylcellulose solution was prepared. 10gm Carboxymethylcellulose were dissolved in 100ml hot distilled water and prepared homogenous solution. CMC solutions are added in the Basal salt medium (Peptone 10gm; KH_2PO_4 0.5gm; $K_2HPO_4.3H_2O$ 0.5gm; $MgSO_4$ 0.5gm; Glucose 40gm; CMC 10gm; Agar 15gm; Distilled water 1000ml). Autoclaved 15 min for 15lbs. Media was pouring in petri dish and allow solidifying. Congored method was used for detection. After 1 hour of incubation, the plates were stained with 0.1% congo red and kept for 30 minutes. The congored was poured off and the plates were washed with 1% sodium chloride. After few min. the background turned pink making the visualization of the zone easier (Hankin and Anagnostakis, 1975; Hankin and Anagnostakis 1976).

Index of Relative enzyme activity

The enzymatic activities were estimated according to the method reported by Hankin and Anagnostakis (1975) who proposed an Index of Relative Enzyme Activity Index (REA). The experiments were performed in triplicate and data was statistically analyzed (Goldbeck *et al*., 2012). Index of Relative Enzyme Activity (Choudhary and Jain, 2012; Rajamani and Hilda, 1987).

$$\text{Clear zone ratios} = \frac{\text{Clear zone diameter}}{\text{Colony diameter}}$$

Growth simulation/inhibition index

Different isolates will be cultured on growth media (malt extract agar; PDA or Czapeks agar) and enzymatic activity test media and observed growth simulation/inhibition index (Khokhar *et al*., 2012).

$$\text{Growth simulation/inhibition index} = \frac{\text{Colony diameter on Enzymatic activity media}}{\text{Colony diameter on control agar}}$$

RESULTS

The isolation and characterization of fungal species from different place of Jabalpur was undertaken in this study. It has been observed that high numbers of fungi were obtained from Ranital region as compared to Civil line of Jabalpur. Total 10 isolates were isolated from MSW was *A. niger*, *A. flavus*, *Aspergillus* sp., *Mucor* sp., *Alternaria alternata*, *A. Glaucus*, *Sarcinella* sp., *A. ustus*, *Cladosporium* sp. and Unidentified sp. (1). *A. niger* and *A. flavus* were most dominating species (Table 1). The isolation and characterization of 18 species of fungi were isolated from different composts, which include the species of fungi viz., *A. niger*, *A. flavus*, *Aspergillus* sp., *A. ustus*, *Mucor* sp., *Alternaria alternata*, *Cladosporium* sp. (Ashraf *et al*., 2007). *Aspergillus* sp. was most dominant genus represented by 5 isolates (Roy *et al*., 2005; Ashraf *et al*., 2007). In waste fungi were also present viz. *A. niger*, *A. flavus*, *Aspergillus* sp., *A. ustus*, *Mucor* sp., *Alternaria alternata*, *Cladosporium* sp. (Ashraf *et al*., 2007) *A. glaucus*, *Sarcinella* sp., Unidentified(1) (Roy *et al*., 2005), *Trichoderma*, *Alternaria*, *Penicillium*, *Ulocladium* and *Aspergillus* (Rebollido *et al*., 2008), *Chaetomium thermophilum*, *Trichoderma viride* and complex microorganisms such as *Trichoderma* sp., white-rot fungi, *Candida rugopelliculosa* (Gautam *et al*., 2010b).

It is important to mention that a large verity of mesophillic, thermotolerent, thermophillic aerobic microorganism, including bacteria, actinomycetes, yeast and various other fungi have been extensively reported in composting (Finstein and Morris, 1975; Strom, 1985; Faure and Deschanps, 1991).

Table 1 Name of isolated fungus in sample A and B

S. No.	Code No.	Name of fungi	Code No.	Name of fungi
1	SA1	*A. niger*	SB1	*Alternaria alternata*
2	SA2	*A. flavus*	SB2	*A. glaucus*
3	SA3	*Aspergillus* sp.	SB3	*Cladosporium* sp.
4	SA4	*Mucor* sp.	SB4	*Sarcinella* sp.
5	-	-	SB5	*A. ustus*
6	-	-	SB6	Unidentified 1sp.
7	-	-	SB7	*A. niger*
8	-	-	SB8	*A. flavus*

Table 2 Screening of fungus based on enzyme activity

S. No.	Name of fungi	Amylase activity	Cellulase activity	Protease activity
1	*A. flavus*	++	++	-ve
2	*A. niger*	-ve	-ve	+
3	*A. glaucus*	++	++	-ve
4	*A. ustus*	++	+	+
5	*Aspergillus* sp.	++	++	-ve
6	*Alternaria alternata*	+	-ve	-ve
7	*Sarcinella* sp.	+	-ve	-ve
8	*Mucor* sp.	++	+	-ve
9	Unidentified sp.(1)	++	++	+
10	*Cladosporium* sp.	-ve	-ve	-ve

Note: (++) excellent, (+) good, (-ve) poor

have been found to exhibit high biodegrading activity due to the secretion of enzyme (Table 2; Fig 1). Fungi released the high amount of enzyme that was responsible for the biodegradation process (Ashraf *et al*., 2007; Chinedu *et al*., 2010). *A. flavus, Aspergillus* sp., *Mucor* sp. (Vyas *et al*., 2005; Roy *et al*., 2005) *A. glaucus, A. ustus,* Unidentified (1) were screen out. Rest of the isolates control grow on media but did not show any enzymatic activity which presumably may be due to the utilization of other materials, which are readily digestible (Lal *et al*., 1996). Index of relative enzyme activity and growth stimulation/inhibition index was observed in amylase activity hydrolysis activity index was zero in *A. niger* and *Cladosporium* sp. and other species give good result unidentified sp 1 followed by *A. glaucus*, *Aspergillus* sp., *Mucor* sp., *A. flavus*, *Alternaria alternata*. Growth stimulation/inhibition index was very good in *Sarcinella* sp. and Unidentified sp., that mean growth was promoted in amylase enzymatic activity media (Table 3). Hydrolysis activity index of cellulase enzyme was maximum in unidentified sp. (1) followed by *Mucor* sp., *Aspergillus* sp. and *A. glaucus* in CMC agar media growth of fungi was stimulated in *Aspergillus* sp. and *Alternaria alternata*.

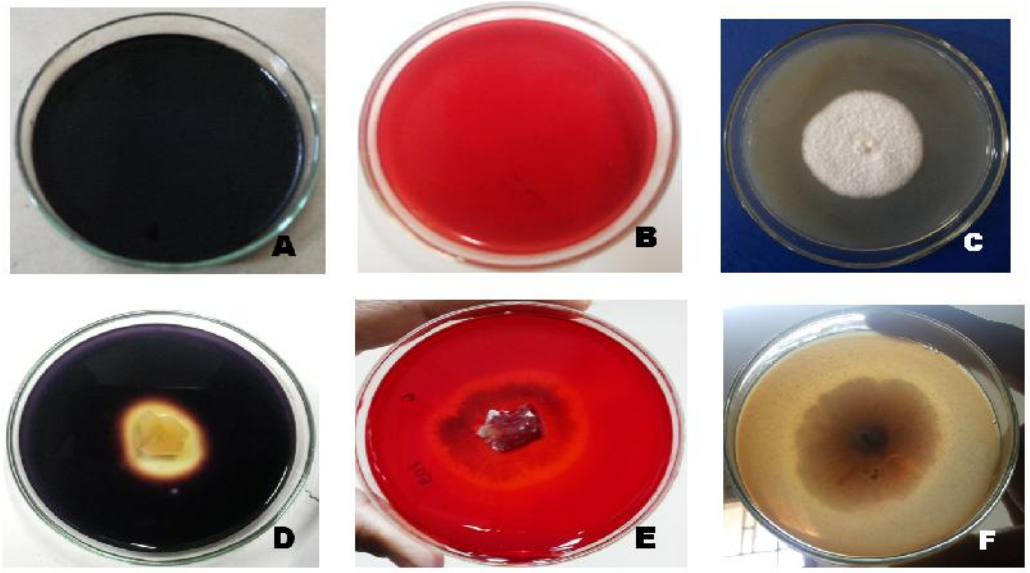

Fig 1 Different enzymatic activity: Control plate of (A) Amylase activity (B) Cellulase activity (C) Protease activity; Zone of positive (D) Amylase activity (E) Cellulase activity (F) Protease activity

These isolates when screened for their different enzymatic activity like cellulase activity through congo red method. It was observed that 6 species of fungi showed positive response. Amylase activity was estimated through starch hydrolysis. It was determined that out of 10 species 8 species of fungi produced amylase enzyme. Proteolytic activity was estimated by casine hydrolysis and only 3 species of fungi were responsible. However, it has been observed that A. *ustus* and Unidentified sp. (1) was gave all assay tests positive. A. *flavus*, A. *glaucus, Aspergillus* sp. and *mucor* sp. showed amylase and cellulase test positive. A. niger gave only protease result positive. *Sarcinella* sp. and *Alternaria alternata* exhibited amylase test positive. The present finding indicates that *A. flavus, Aspergillus* sp., *Mucor* sp., *A. glaucus, A. ustus* and Unidentified sp. (1) showed significant result for enzyme production. Among the tested fungi *A. flavus, Aspergillus* sp., *A. ustus, Mucor* sp., *A. glaucus, Sarcinella* sp., Unidentified (1)

In other fungi, colony diameter was decrease in CMC medium (Table 4). Index of relative enzyme activity for protease enzyme only 3 fungal isolates *A. ustus* followed by Unidentified sp (1) and *A. niger*. Growth stimulation/inhibition index was maximum in *Cladosporium* sp. followed by *A. flavus*, *Aspergillus* sp. and *Mucor* sp. (Table 5).

In the present study, it was observed that only *A. ustus* and unidentified sp (1) give positive result in all enzymatic activity. These were able to show good hydrolysis activity index (ICMC) for amylase, cellulase and proteases activity. In all enzymatic medium growth of *A. ustus* was inhibition that mean for better enzyme production from *A. ustus.*

Table 3 Index of Relative enzyme activity and Growth stimulation/inhibition index of Amylase enzyme

S. No.	Fungal isolates	Hydrolysis Zone Diameter [cm]	Colony diameter on starch hydrolysis agar [cm]	Hydrolysis Activity index (ICMC)	Colony diameter on control agar [cm]	Growth stimulation/ inhibition index
1	*A. flavus*	5.9	5.43	1.086	6.1	0.89
2	*A. niger*	0.000	4.86	0.000	5.8	0.837
3	*A. glaucus*	5.21	4.13	1.261	4.6	0.897
4	*A. ustus*	4.87	4.7	1.036	5.1	0.921
5	*Aspergillus* sp.	3.1	2.6	1.1923	2.9	0.896
6	*Alternaria alternata*	3.9	3.6	1.083	4.1	0.878
7	*Sarcinella* sp.	6	5.83	1.029	4.5	1.295
8	*Mucor* sp.	6.1	5.6	1.089	6.7	0.835
9	Unidentified sp.(1)	4.36	3.4	1.282	2.9	1.172
10	*Cladosporium* sp.	0.000	4.03	0.000	4.1	0.982

Table 4 Index of Relative enzyme activity and Growth stimulation/inhabitation index of cellulase enzyme

S. No.	Fungal isolates	Hydrolysis Zone diameter[cm]	Colony diameter on CMC agar [cm]	Hydrolysis Activity index (ICMC)	Colony diameter on control agar [cm]	Growth stimulation/ inhibition index
1	*A. flavus*	6.21	5.76	1.078	6.1	0.944
2	*A. niger*	0.000	5.2	0.000	5.8	0.896
3	*A. glaucus*	4.76	4.2	1.133	4.6	0.913
4	*A. ustus*	5.2	4.8	1.083	5.1	0.941
5	*Aspergillus* sp.	4.21	3.7	1.13	2.9	1.275
6	*Alternaria alternata*	0.000	4.65	0.000	4.1	1.134
7	*Sarcinella* sp.	0.000	3.86	0.000	4.5	0.857
8	*Mucor* sp.	3.24	2.8	1.157	6.7	0.417
9	Unidentified sp.(1)	2.12	1.5	1.413	2.9	0.517
10	*Cladosporium* sp.	0.000	3.2	0.000	4.1	0.780

Table 5 Index of Relative enzyme activity and Growth stimulation/inhabitation index of protease enzyme

S. No.	Fungal isolates	Hydrolysis zone Diameter [cm]	Colony diameter on Protease activity agar [cm]	Hydrolysis Activity Index (ICMC)	Colony diameter on control agar [cm]	Growth stimulation/ inhibition index
1	*A. flavus*	0.000	6.4	0.000	6.1	1.049
2	*A. niger*	2.68	2.56	1.0468	5.8	0.441
3	*A. glaucus*	0.000	3.2	0.000	4.6	0.695
4	*A. ustus*	5.91	5.06	1.167	5.1	0.992
5	*Aspergillus* sp.	0.000	3.03	0.000	2.9	1.044
6	*Alternaria alternata*	0.000	5.4	0.000	4.1	0.8450
7	*Sarcinella* sp.	0.000	4.21	0.000	4.5	0.9355
8	*Mucor* sp.	0.000	6.83	0.000	6.7	1.019
9	Unidentified sp.(1)	2.87	2.53	1.134	2.9	0.872
10	*Cladosporium* sp.	0.000	4.8	0.000	4.1	1.17

DISCUSSION

The soil is a dynamic medium for microbial/biological activities and that the number and kind of microorganisms present in a particular soil depends on the environmental factors such as amount and type of available nutrients, moisture and the degree of aeration, pH, temperature etc (Prescott *et al*., 1993). They play pivotal role in various biochemical processes and thus are responsible for recycling of organic compounds in nature. Fungi are an important component of the microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bisby, 1995). The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem. They perform ecological services that strongly impact the quality of human life and have enormous potential for providing economic benefits. It is estimated that there are 1.5 million fungal species on earth, of which only 70,000 have been described up to now thus presents a potential source of novel organisms (Hawksworth and Rossman, 1997). They grow and carry out active metabolism when conditions are favorable with adequate moisture, aeration and relatively high concentration of utilizable substrates (Miyanoto *et al*., 2002).

The data indicated the dominance of genus *Aspergillus* in the MSW of surveyed habitats. Members of *Aspergillus* have been reported widely in soil since their nature allows them to have nutrients and moisture for all different studies of life cycle. The published work on global distribution of *Aspergilli* in soil has been documented by Domesch *et al*. (1980), Raper and Fennel (1965). MSW was dumped in soil so number of Aspergilli was also present in MSW.

Among fungi species of certain genera such as *Aspergillus, Penicillium, Paecilomyces, Rhizopus* and *Rhizomucor* are well-know producers of proteases (Vamsi Krishna *et al*., 2009). Protease has an important role to decompose protein substance (Sunantapongsuk, 2006). Some MSW isolates *Alternaria alternata*, *Alternaria* sp., *Acremonium butyri*, *A. clavatus*, *A. flavus*, *A. candidus*, *A. luchuensis*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, *Aspergillus* sp., *Chaetomium* sp., *Chrysosporium* sp., *Cladosporium* sp., *Curvularia lunata*, *Curvularia* sp., *Drechslera* sp., *Fusarium oxysporum*, *Fusarium roseum*, *Gliocladium* sp., *Humicola* sp., *Mucor* sp., *Myrothecium* sp., *Paecilomyces* sp., *Penicillium digitatum*, *Penicillium* sp., *Rhizopus* sp., *Sclerotium rolfsii*, *Trichoderma viride*, *Trichoderma* sp., *Verticillium* sp., were able to show positive cellulase enzyme activity test (Gautam *et al*., 2012).

Fungi have unique properties, which secrete different extracellular enzyme (cellulase, amylase, proteases, laccase etc.) and breakdown larger molecule to smaller molecule. Now

a day's different worker isolated fungi from different sources (Soil, Air, Water, MSW, municipal sewage water, Vegetable, fruits degraded land etc.) and check different enzymatic activities to reduce the cost of product and easy. This study generated information regarding waste decomposition by fungi disposal of solid waste. For removal of heavy metal and bioconversion of MSW and Municipal sewage water by fungi work was carried out by different worker Verma and Jamaluddin, 2012a; Verma and Jamaluddin, 2012b; Verma *et al*., 2012; Chandraka *et al*., 2012. The techniques so developed can be utilized by the user agencies.

CONCLUSION

It has been observed that fungi out of 10 strains *A. niger, A. flavus, Aspergillus* sp., *A. ustus, Moucr* sp., *Alternaria alternata, Cladosporium* sp., *A. Glaucus, Sarcinella* sp. and Unidentified 1 sp. were found to be most potential candidate to degrade solid waste. Further investigations are required to proper application of these organisms for cellulase, protease and amylase production beneficial for bioconversion of MSW. Bioconversion is one of the more economical and environmentally safe methods of recycling waste generated by the consumer society. The techniques so developed can be utilized by the user agencies.

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