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Screening of Cellulase Production by Fungal Isolates from Rhizosphere Region of Mine Degraded Land in Dalli-Rajhara (Chhattishgarh)

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Abstract—*The main objective of this study is to screen potential of fungi which was able to secret cellualse enzyme, maximize economic benefits and at the same time protection of environment by microbial activity for restoration of degraded land. Cellulase are group of hydrolytic enzymes capable of hydrolyzing the most abundant organic polymer i.e. Cellulose to smaller sugar components including glucose subunits. Quantitative method was used for screening screen potential of fungi. After 5 days of incubation clear zone around fungal colonies were able to observe with the help of stain Congo-red. Out of 38 test fungi 15 was able to give clear zone. Highest clear zone diameter was observed in Periconia hispidula (1.5cm), Mucor hiemalis (1cm) and minimum was observed in Alternaria alternata (0.27) and Cladosporium oxysporum (0.28cm). Screened fungi were used as a biofertilizer, for increasing soil fertility and restored the degraded land.*

Keyword*: rhizosphere fungi, hydrolysis zone, Iron ore, mining, soil fertility*

1. INTRODUCTION

Mining provides the materials required to sustain quality of life but exerts a long lasting impact on landscape, eco-system and socio-cultural-economic considerations (Sahu and Dash, 2011). Without mining life is tasteless (Panda and Barik, 2010). Opencast mining, damage the top soil layer and its effect caused several changes in physical, chemical and microbiological properties of soil (Kundu and Ghose, 1998). India is a developing country and produces as many as 84 minerals comprising 4 fuels, 11 metallic, 49 non-metallic industrial and 20 minor minerals (CPCB, 2007) by open cast mining. Open cast mining creates more pollution as they generate much higher quantities of waste as compared to the underground mining. Soil fertility was increase and maintained by microorganism (Verma and Verma, 2016) by secretion of extracellular enzyme on plant dead material. Plants residues are a rich source of cellulose (Hameeda *et al*., 2006). As the main component of plant fiber structures, cellulose is arranged in crystalline to amorphous forms and is a substrate to numerous species of both fungi and bacteria relying on extracellular enzymes. The most studied group of cellulose-degrading microorganisms is the fungi, which are characterized by multi-component, synergistic cellulolytic enzyme systems (Berg and Laskowski, 2006). Fungal cellualse have proved to be a better candidate than other microbial cellulases, with their secreted free cellulase complexes comprising all three components of cellulase (Wood et al., 1988). Cellulolytic enzyme systems are extensively studied of many microorganisms, like complexed or non-complexed, including aerobic and anaerobic bacteria, white rot and soft rot fungi and anaerobic fungi. Cellulase is mostly secreted as free molecules by filamentous fungi, actinomycetes and in aerobic bacteria, (Bayer *et al.*, 1999; Gilkes *et al.*, 1989; Mathew *et al.*, 2008). Cellulase play an important role in carbon availability and so can be-used to give a preliminary indication of some of the physical chemical properties of soil, thus, easing agricultural soil management strategies (Ndakidemi and Makoi, 2008). In the present study we screen non dominated fungi form iron ore mine overburden dumps.

2. MATERIALS AND METHODS

2.1 Study Site

Dalli-Rajhara is located on a hill range bounded by 20°33'0" and 20°34'30" N latitude and 81°1'0" and 81°4'30" E longitude under Balod (District) in Chhattisgarh. The climate of Balod is tropical and the whole year is divisible in to three well marked seasons viz., summer (March to June), monsoon (July to October) and winter (November to February). Temperature rises up to even 47ºC in the month of May/June and comes down to 6.5ºC in December/January. The mean annual rainfall is 1400 mm, 75-80% of which occurs during the monsoon period.

2.2 Soil vegetation

The vegetation of natural forest is dominated by teak (*Tectona grandis*) and other tree constituents of vegetation is consisting of *Acacia nilotica*, *Aegle marmelos*, *Acacia catechu*, *Albizia procera*, *Bauhinia retusa*, *Boswellia serreta*, *Butea monosperma*, *Dalbergia paniculata*, *Diospyros melanoxylon*, *Dendrocalamus strictus*, *Pongamia pinnata*, *Shorea robusta*, *Terminalia tomentosa*, and *Ziziphus mauritiana* (Banerjee *et al*., 1997).

2.3 Soil sampling, isolation of fungi and Identification of Fungi

Soil sample were collected from planted trees in iron ore mined overburden dumps. Sample uses for fungal quantification were taken from rhizosphere zone by removing one cm soil from surface. A soil auger used which as washed thoroughly before starting of sampling procedure. Sampling done in 10-20 cm depth in soil horizon and carefully collected in polyethylene bags and their mouth were tied with rubber bands. In lab sample were homogenized and spread on paper to remove plant material, they are air dried, sifted with 2mm mesh sieve and stored at 4ºC used for experiment (Parkinson, 1979). Dilution was prepared and 1 ml of the sample was placed in a sterile Petri-dish and 10 ml of sterile cooled (40°C) PDA was added. The contents were thoroughly mixed and the plates were incubated (Warcup, 1950). Incubate the plate at 27ºC for 3 to 7 days in BOD incubator.

After incubation distinct colonies were identified. The cultures were identified on the basis of macroscopic (colonial morphology, color, texture, shape, diameter and appearance of colony) and microscopic characteristics (spore bearing fruiting body, spore size, growth rate hyphae, septation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium). Pure cultures of fungi isolates were identified with the help of literature (Gilman, (1957); Barnett, (1962); Booth, (1971); Ellis, (1971); Barnett and Hunter, (1972); Booth, (1977); Nagmani *et al*., (2006); Verma *et al*., (2008) and Expert in Forest Pathology Division, T.F.R.I., Jabalpur were referred for identification of fungi. After the identification pure culture was stored in refrigerator for further use and preservation.

2.4 Screening by enzymatic assay of isolated soil fungi

Prepared the Basal salt medium (for cellulase activity), 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 (Hankin and Anagnostakis, 1977) then incubated plate for 72-96 hours at 25ºC in an inverted position. After incubation flood the surface of basal salt medium with 1% Congo red dye (30 min), followed by de-staining with 1 M NaCl solution for 20 min. clear zones could be observed only around colonies of the active fungal strains.

2.5 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) (Goldbeck *et al*., 2012; Choudhary and Jain, 2012; Bradner *et al*., 1999; 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 (Potato Dextrose Agar) and enzymatic activity test media and observed growth simulation/inhibition index (Bradner *et al*., 1999).

$$\text{Growth simulation/ inhibition index} = \frac{\text{Colony diameter on basal salt media}}{\text{Colony diameter on potato dextrose agar}}$$

3. RESULT AND DISCUSSION

Over all 92 fungi were isolated from rhizosphere region of different planted trees. Among 92 fungi 27 are belonging to generas i.e. *Absidia*, *Acremonium*, *Alternaria*, *Aspergillus*, *Biopolarus*, *Botryotrichum*, *Cephalosporium*, *Cladosporium*, *Clamydomyces*, *Curularia*, *Emericella*, *Eupenicillum*, *Fusarium*, *Gliocladium*, *Memmoniella*, *Mucor*, *Nigrospora*, Non sporolating hypomyctes, *Oidiodendron*, *Paecilomyces*, *Penicillum*, *Periconia*, *Phoma*, *Scytalidium*, Sterile fungi, *Trichoderma* and *Tritriachium* for check their ability to degrade cellulose. While, study only some common species were selected to study enzymatic study. During study 4 genera like *Aspergillus*, *Fusarium*, *Penicillum*,and *Trichoderma* were most dominated in iron ore mine soil. Out of 92 fungal species 38 were common in iron ore mine soil.

Total 15 fungal species were given the hydrolysis zone around the colonies, which was grown in Basel salt agar medium supplemented with CMC as a sole sources of cellulose. Highest REA was observed in *Periconia hispidula* (1.592) followed by Non sporolating hypomyctes (1.464), *Mucor hiemalis* (1.309) and lower REA was recorded in *Phoma* sp. (0.915) followed by *Alternaria alternata* (1.049) and *Absidia fuca* (1.073).

Growth simulation/inhibition index was computed as the colony diameter on carboxymethyl agar/colony diameter on control agar ratio. The index value <1, represented substrate inhibited fungal growth, while the index value >1, exhibited substrate rendered growth stimulation. The hydrolysis zone diameter compare with the colony diameter on carboxymethyl agar medium. However, hydrolysis zone diameters were not

greater than colony diameter in case some isolates. Growth stimulation index ranges between 1.897 and 1.016. Therefore, hydrolysis activity indices were found to be >1 in case of 11 fungi such as Sterile fungi 7, *Absidia fuca*, *Tritriachium dependens*, Sterile fungi 8, *Oidiodendron mais*, *Cephalosporium indicum*, Sterile fungi 3, *Cladosporium variabile*, *Biopolarus halodus*, *Clamydomyces palmarum*, *Eupenicillum* sp. 1, *Phoma* sp. 1. Growth was slightly reduced in 7 fungi. Some colony was show < 1, Range of inhibition index was 0.791-0.393. Total 14 fungi were appearing in these ranges.

Verma and Verma, (2016) also isolated 54 dominated fungi (*Aspergillus*, *Penicillum*, *Fusarium* and *Trichoderma*) from iron ore mined overburden dumps and observed 26 positive result. Verma *et al*., (2015) isolated 10 fungi and observed 6 fungi was secreted high amount of enzyme such as *A. flavus*, *Aspergillus sp.*, *A. glaucus*, *A. ustus*, *Mucor sp.*, Unidentified sp. (1). Fungi *Emericella nidulans*, *Emericella echinulata*, *Absidia corymbifera*, *Myrothecium verrucaria*, *Cunninghamella echinulata*, *Circinella muscae*, *Botryotrichum piluliferum*, *Ulocladium* sp., *Drechslera* sp., *Emericella dentate*, *Geotrichum candidum*, *Mucor fuscus*, *Trichurus spiralis*, *Curvularia* sp., *Alternaria alternate*, *Emericella stellatus* isolated from soil and give positive cellulase activity (Afifi, 2013). Reddy et al, (2014) observed highest cellulase producing isolate was *Aspergillus niger*, *Aspergillus flavus* and the least was *Trichoderma sp.* The most common and most effective cellulose producers are *Trichoderma ressei*, *T. koningii*, *Fusarium* sp., *Aspergillus* and *Penicillium* sp., (Yalpani 1987; Gautam et al., 2010).

Cellulases have enormous potential in industrial applications (Walsh, 2002; Gautam *et al.*, 2012), food processing, feed preparation, waste-water treatment, detergent formulation, textile production and in other areas. Additional potential applications include the production of wine, beer and fruit juice, biofuel (Philippidis, 1994), use as biofertilizer (Verma and Verma, 2016).

4. CONCLUSION

From the current study concluded that degree of variability of enzyme production by the fungi isolated from iron ore mine overburden dumps. This indicates that the enzyme production differs between fungi and often corresponds to the requirements of its habitat. According to sources of isolated fungi screened fungi was used for different purpose.

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Table 1: Growth and cellulase enzyme activity of fungal isolates

S. No.	Fungal isolates	HZ (cm)	CD (cm)	REA	CDP (cm)	GS/ II
1	*Absidia fuca*	5.72	5.33	1.073	3.23	1.65
2	*Acremonium strictum*	3.16	2.48	1.274	3.28	0.756
3	*Alternaria alternata*	5.83	5.56	1.049	6.32	0.879
4	*Alternaria tenuissima*	0	4.56	0	4.74	0.962
5	*Biopolarus halodus*	4.14	3.76	1.101	3.53	1.065
6	*Botryotrichum piluliferum*	3.03	2.73	1.109	4.74	0.576
7	*Cephalosporium indicum*	0	4.43	0	3.15	1.406
8	*Cladosporium oxysporum*	2.63	2.35	1.119	4.57	0.514
9	*Cladosporium variabile*	4.03	3.73	1.08	3.23	1.154
10	*Clamydomyces palmarum*	3.53	3.03	1.165	2.94	1.03
11	*Curularia indica*	0	5.33	0	6.15	0.867
12	*Emericella nidulans*	0	3.55	0	4.37	0.812
13	*Eupenicillum* sp.	5.16	4.43	1.165	4.35	1.018
14	*Gliocladium deliquescens*	0	3.36	0	3.43	0.979
15	*Memmoniella echinata*	0	2.03	0	5.16	0.393
16	*Mucor hiemalis*	3.98	2.98	1.309	6.32	0.471
17	*Nigrospora oryzae*	0	3.06	0	4.25	0.72
18	*Nigrospora padwicki*	0	6.03	0	6.17	0.977
19	*Nigrospora panici*	0	5.06	0	6.39	0.791
20	Non sporolating hypomyctes	2.24	1.53	1.464	2.88	0.531
21	*Oidiodendron mais*	0	3.63	0	2.56	1.418
22	*Paecilomyces lilacinus*	3.84	3.16	1.215	3.75	0.843
23	*Periconia hispidula*	4.03	2.53	1.592	4.83	0.524
24	*Phoma* sp.	2.16	2.36	0.915	5.74	0.411
25	*Phoma* sp. 1	0	4.93	0	4.95	0.996
26	*Scytalidium lignicola*	0	3.08	0	3.22	0.957
27	*Scytalidium thermophilum*	6.43	5.86	1.097	5.93	0.988
28	Sterile fungi 1	0	3.69	0	4.37	0.844
29	Sterile fungi 2	0	4.1	0	3.14	1.306
30	Sterile fungi 3	0	3.32	0	4.66	0.712
31	Sterile fungi 4	0	1.09	0	2.68	0.407
32	Sterile fungi 5	0	4.36	0	4.97	0.877
33	Sterile fungi 6	0	4.23	0	2.23	1.897
34	Sterile fungi 7	0	4.96	0	3.21	1.545
35	Sterile fungi 8	0	4.36	0	4.73	0.921
36	Sterile fungi 9	0	3.6	0	4.67	0.771
37	Sterile fungi 10	0	2.76	0	3.63	0.76
38	*Tritriachium dependens*	0	5.63	0	3.64	1.547

REA=Relative enzyme activity index (values more than 0 showed positive cellulase enzymatic activity); HZ=Hydrolysis zone; CD=Colony diameter; CDP=Colony diameter on potato dextrose agar; GS/ I I=Growth simulation/inhibition index