ISSN (Online): 2349-1183; ISSN (Print): 2349-9265
TROPICAL PLANT RESEARCH
The Journal of the Society for Tropical Plant Research
5(3): 396–404, 2018
DOI: 10.22271/tpr.2018.v5.i3.049

Research article

Qualitative estimation of amylase enzyme activity of fungal species isolated from iron ore mined overburden soil

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Abstract: The enzyme is a biocatalyst and processes many biological activities. In the present investigation soil samples were collected from iron ore mined overburden soil and fungal flora were isolated. For qualitative estimation of fungi for amylase activity, plate assay method was used. Out of 99 fungi, only 21 test fungi were found to produce amylase. Most of the amylase producers identified belonged to *Aspergillus* sp., *Penicillium* sp., *Periconia* sp., *Scytalidium* sp., *Memmoniella* sp., *Trichoderma* sp., *Phoma* sp. and *Fusarium* sp. followed by *Alternaria* sp. Other 78 test fungi were found to grow on medium, but were unable to produce amylase. Maximum 44 fungi were isolated from Trichocomaceae family and one fungus from Chaetomiaceae and Myxotrichaceae family. Isolated fungi of Chaetomiaceae, Mucoraceae, Mycosphaerelleaceae, Myxotrichaceae and Nectriaceae family were unable to produce enzyme. Maximum numbers of enzyme producing fungi belongede to Trichocomaceae family, followed by Incertae sedis. In the present investigation observed that *Penicillium* sp. 1 give the highest relative enzyme activity index.

Keywords: Chhattisgarh - Degraded land - Dalli-Rajhara - Fungal family - Relative enzyme activity index - Soil fertility.

[Cite as: Verma P & Verma RK (2018) Qualitative estimation of amylase enzyme activity of fungal species isolated from iron ore mined overburden soil. *Tropical Plant Research* 5(3): 396–404]

INTRODUCTION

The fungi present in mine overburden soil have the capacity to tolerate the unfavorable condition, by improving some special character like biosorption of heavy metal, secretion of enzyme etc., and these fungi degrade the plant material and help in improving biogeochemical cycle (Verma *et al*. 2015a, Verma *et al*. 2017). The group of fungi has for a long time been known to excrete extracellular enzyme capable of breaking down complex substrate such as proteins, starch, cellulose and lipids (Barbesgaard 1992). Fungi are sources of amylolytic enzymes suitable for the industrial conversion of starch into maltose or glucose. But the proof is required that these fungi are physiologically capable of breaking down and utilizing the complex polysaccharides of plant cell walls.

Amylase (α-amylase or α-1,4 glucan glucanohydrolase; EC 3.2.1.1) are enzymes that break down starch or glycogen (Mishra & Maheshwari 1996). Amylase enzyme can be derived from several sources, such as plants, animals and microorganisms. Microorganism secretes amylase to the outside of their cells to carry out extra-cellular digestion. These enzyme breakdowns the plant debris in soil and converted into soluble starch.

Amylase constitutes a class of industrial enzymes having approximately 25% of the enzyme market (Sidhu *et al*. 1997, Rao *et al*. 1998). Amylase have potential application in a number of industrial processes such as in the textiles (12%), starch (11%), baking (8%), animal feed (6%), ethanol, food, paper industries, bread making, glucose and fructose syrups, detergents (37%), fuel ethanol from starch, fruit juices, alcoholic beverages, sweeteners**,** digestive aid and spot remover in dry cleaning which use about 75% of industrially produced enzymes (Kang & Cottrell 1979, Tiwari *et al*. 2015). The estimated value of the world market is presently about US$ 2.7 billion and is estimated to increase by 4% annually through 2012 (Das *et al*. 2011). The aim of this

Received: 10 July 2018 Published online: 31 December 2018
https://doi.org/10.22271/tpr.2018.v5.i3.049

study is to isolate and identify the fungal flora in iron ore mine overburden soil and determine the qualitative measurement of amylolytic activity by plate assay method.

MATERIALS AND METHODS

Study site

Dalli-Rajhara is located on a hill range bounded by 20º 33'0" and 20º 34'30" N latitude and 81º 1'0" and 81º 4'30" E longitude under Balod district in Chhattisgarh.

Soil sampling, isolation and identification of fungi

Soil samples were collected from rhizosphere of planted trees in iron ore mined overburden dump. Sample used for fungal quantification were taken from rhizosphere zone by removing one cm soil from the surface. A soil auger was used, which was washed thoroughly before starting of the sampling procedure. Samples were collected from 10–20 cm depth and were carefully placed in polyethylene bags and their mouth were tied with rubber bands. In the laboratry, soil samples were homogenized and spread on paper to remove plant material, they were air dried, and stored at 4ºC for further experiment (Parkinson 1979). Soil dilution was prepared and 1 ml of the sample was placed in a sterile Petri-dish and 10 ml of sterile cooled (40ºC) PDA was added, mixed and the plates were incubated at 27ºC for 3 to 7 days in BOD incubator (Warcup 1950).

After incubation distinct colonies were identified. The cultures were identified on the basis of macroscopic and microscopic characteristics. Pure cultures of fungi isolates were identified with the help of literature (Gilman 1957, Booth 1971, 1977, Ellis 1971, 1976, Barnett & Hunter 1972, Nagmani *et al*. 2006, Verma *et al*. 2008). After the identification pure culture was stored in the refrigerator for further use and preservation.

Screening by enzymatic assay of isolated soil fungi

Prepared the starch agar medium (Starch 20 g; Peptone 5 g; Beef extract 3 g; Agar 15 g; D/W 1000 ml), poured into the sterile Petri dishes. Using needle a bit of the fungal culture (4 mm) cut by cock borer was taken and placed onto the center of media without further disturbing its position. The labeled plate then incubated plate for 72–96 hours at 25ºC in an inverted position. After incubation flood the surface of the starch agar medium plates with iodine solution with a dropper for 30 seconds and pour off the excess iodine solution. The starch hydrolysis around the line of growth of each organism, *i.e.* the colour changes of the medium because amylase is a starch hydrolyzing enzyme (Ross 1976).

Index of relative enzyme activity

The enzymatic activities were estimated according to Hankin & Anagnostakis (1975) method and results were concluded by Relative Enzyme Activity Index (REA) (Goldbeck *et al*. 2012, Choudhary & Jain 2012).

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

Growth simulation/inhibition index

Different isolates were cultured on growth media (Potato Dextrose Agar) and enzymatic activity test media and observed growth simulation/inhibition index (Bradner *et al*. 1999).

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

RESULT AND DISCUSSION

138 soil samples were collected in replication from the different plantation and natural soil. A total of 99 fungal forms were obtained from the samples.

Screening of amylase producing fungi

Out of 99 fungi, only 21 test fungi were found to produce amylase enzyme as indicated by the production of the zone on starch agar medium around the fungal colonies grown at pH 7 after 5 days of incubation at 28±3°C. Most of the amylase producers identified belonged to *Aspergillus* sp., *Penicillium* sp., *Periconia* sp., *Scytalidium* sp., *Memmoniella* sp., *Trichoderma* sp., *Phoma* sp. and *Fusarium* sp. followed by *Alternaria* sp. However, some isolates of *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Phoma* sp., *Scytalidium* sp. and *Trichoderma* sp. did not show any amylase activity. Other 78 test fungi *Absidia* sp., *Acremonium* sp., *Biopolarus* sp., *Botryotrichum* sp., *Cephalosporium* sp., *Cladosporium* sp., *Clamydomyces* sp., *Curularia* sp., *Emericella* sp., *Eupenicillium* sp., *Gliocladium* sp., *Mucor* sp., *Nigrospora* sp., Non sporolating hypomyctes, *Oidiodendron* sp., *Paecilomyces* sp., *Rhizopus* sp., *Verticillum* sp. and Sterile fungi followed by *Tritriachium* sp. were found to grow on medium (pH 7) but were unable to produce zone of hydrolysis. Results presented in table 1 indicate that highest amylase activity was detected in only seven isolates *viz. Penicillium* sp. 1 (0.93 cm),

Aspergillus fumigatus (0.6 cm), *Periconia hispidula* (0.59 cm), *Aspergillus* sp. 3 (0.57 cm), *Penicillium citreonigrum* (0.52 cm), *Penicillium roseopurpureum* (0.47 cm), *Memmoniella echinata* (0.42 cm), *Phoma* sp. (0.42 cm), *Aspergillus parasiticus*, *Aspergillus terreus*, *Penicillium funiculosum*, *Scytalidium lignicola*, *Trichoderma polysporum* (0.4 cm). The enzymatic activity was considerably low in other fungi such as *Aspergillus nidulars* var. *echinulatus*, *Fusarium chlamydosporum* (0.3), *Penicillium rugulosum* (0.29), *Aspergillus* sp. 4 (0.27), *Aspergillus janus* (0.23), *Aspergillus versicolor* (0.2), *Alternaria alternata* (0.17) and *Aspergillus awamori* (0.1).

Table 1. Growth and amylase enzyme activity of Fungal isolates.

S.N.	Fungal isolates	Family	HZ (cm)	CD (cm)	REA	CDP (cm)	GS/ II
1	*Absidia fuca*	Mucoraceae	0	6.36	0	3.22	1.976
2	*Acremonium strictum*	Nectriaceae	0	2.63	0	3.83	0.687
3	*Alternaria alternata*	Pleosporaceae	6.23	6.06	1.028	6.32	0.959
4	*Alternaria humicola*	Trichocomaceae	0	3.86	0	4.94	0.781
5	*Alternaria tenuissima*	Trichocomaceae	0	7.03	0	4.75	1.48
6	*Alternaria awamori*	Trichocomaceae	3.56	3.46	1.029	4.95	0.699
7	*Alternaria candidus*	Trichocomaceae	0	2.63	0	4.36	0.603
8	*Alternaria clavatus*	Trichocomaceae	0	4.44	0	3.35	1.325
9	*Alternaria flavus*	Trichocomaceae	0	7.06	0	4.32	1.634
10	*Alternaria flavus* var. *columnaris*	Trichocomaceae	0	4.46	0	3.17	1.407
11	*Alternaria flavus* var. *oryzae*	Trichocomaceae	0	2.85	0	4.67	0.61
12	*Alternaria fumigatus*	Trichocomaceae	3.33	2.73	1.219	7.27	0.376
13	*Alternaria humicola*	Trichocomaceae	0	4.29	0	4.98	0.861
14	*Alternaria janus*	Trichocomaceae	2.86	2.63	1.087	4.74	0.555
15	*Alternaria nidulars* var. *echinulatus*	Trichocomaceae	6.16	5.86	1.051	4.46	1.313
16	*Alternaria niger*	Trichocomaceae	0	5.36	0	4.74	1.13
17	*Alternaria parasiticus*	Trichocomaceae	3.26	2.86	1.139	5.74	0.498
18	*Alternaria repens*	Trichocomaceae	0	5.06	0	4.83	1.048
19	*Alternaria restrictus*	Trichocomaceae	0	4.36	0	3.86	1.129
20	*Aspergillus* sp. 1	Trichocomaceae	0	6.54	0	5.46	1.198
21	*Aspergillus* sp. 2	Trichocomaceae	0	2.76	0	4.67	0.591
22	*Aspergillus* sp. 3	Trichocomaceae	5.73	5.16	1.11	4.26	1.211
23	*Aspergillus* sp. 4	Trichocomaceae	5.33	5.06	1.053	5.22	0.969
24	*Aspergillus sydowii*	Trichocomaceae	0	7.66	0	5.15	1.487
25	*Aspergillus terreus*	Trichocomaceae	3.93	3.53	1.113	4.24	0.832
26	*Aspergillus unguis*	Trichocomaceae	0	6.83	0	4.92	1.389
27	*Aspergillus versicolor*	Trichocomaceae	4.36	4.16	1.048	5.67	0.733
28	*Biopolaris halodus*	Pleosporaceae	0	4.83	0	3.56	1.357
29	*Botryotrichum piluliferum*	Chaetomiaceae	0	2.96	0	4.74	0.624
30	*Cephalosporium indicum*	Incertae sedis	0	6.57	0	3.16	2.079
31	*Cladosporium oxysporum*	Mycosphaerelleaceae	0	3.36	0	4.77	0.704
32	*Cladosporium variabile*	Mycosphaerelleaceae	0	4.43	0	3.25	1.363
33	*Clamydomyces palmarum*	Incertae sedis	0	3.86	0	2.96	1.304
34	*Curularia indica*	Pleosporaceae	0	3.83	0	6.15	0.623
35	*Emericella nidulans*	Trichocomaceae	0	2.63	0	4.34	0.605
36	*Eupenicillium* sp.	Trichocomaceae	0	6.56	0	7.12	0.921
37	*Fusarium oxysporum*	Nectriaceae	0	6.13	0	5.36	1.144
38	*Fusarium chlamydosporum*	Nectriaceae	5.66	5.36	1.056	7.63	0.702
39	*Fusarium javanicum*	Nectriaceae	0	4.65	0	4.92	0.945
40	*Fusarium moniliforme*	Nectriaceae	0	5.73	0	5.75	0.997
41	*Fusarium poae*	Nectriaceae	0	2.16	0	3.16	0.683
42	*Fusarium roseum*	Nectriaceae	0	5.14	0	6.38	0.752
43	*Fusarium* sp.	Nectriaceae	0	5.43	0	4.81	1.129
44	*Fusarium solani*	Nectriaceae	0	3.87	0	3.26	1.187
45	*Fusarium udum*	Nectriaceae	0	5.56	0	4.65	1.195
46	*Gliocladium deliquescens*	Hypocreaceae	0	2.83	0	3.43	0.825
47	*Memmoniella echinata*	Incertae sedis	3.26	2.84	1.148	5.11	0.556
48	*Mucor hiemalis*	Mucoraceae	0	5.76	0	6.56	0.878
49	*Nigrospora oryzae*	Hypocreaceae	0	7.36	0	4.27	1.724
50	*Nigrospora padwicki*	Hypocreaceae	0	6.33	0	6.17	1.025
51	*Nigrospora panici*	Hypocreaceae	0	5.47	0	6.39	0.856

52	Non sporolating hypomyctes	-	0	6.45	0	2.83	2.279
53	*Oidiodendron mais*	Myxotrichaceae	0	5.43	0	2.56	2.121
54	*Paecilomyces lilacinus*	Trichocomaceae	0	2.56	0	3.75	0.682
55	*Penicillium adametzi*	Trichocomaceae	0	2.63	0	2.54	1.035
56	*Penicillium asperum*	Trichocomaceae	0	3.56	0	3.75	0.949
57	*Penicillium aurantiogriseum*	Trichocomaceae	0	3.21	0	2.71	1.185
58	*Penicillium citreonigrum*	Trichocomaceae	3.14	2.62	1.198	5.14	0.509
59	*Penicillium citrinum*	Trichocomaceae	0	3.65	0	4.32	0.845
60	*Penicillium commune*	Trichocomaceae	0	2.87	0	3.92	0.732
61	*Penicillium funiculosum*	Trichocomaceae	5.26	4.86	1.082	4.98	0.975
62	*Penicillium nigricans*	Trichocomaceae	0	3.16	0	3.16	0
63	*Penicillium novae*	Trichocomaceae	0	3.98	0	3.84	1.036
64	*Penicillium oxalicum*	Trichocomaceae	0	6.13	0	4.25	1.442
65	*Penicillium restrictum*	Trichocomaceae	0	5.53	0	5.27	1.049
66	*Penicillium roseopurpureum*	Trichocomaceae	3.93	3.46	1.135	5.26	0.658
67	*Penicillium rugulosum*	Trichocomaceae	4.84	4.55	1.063	5.64	0.807
68	*Penicillium* sp. 1	Trichocomaceae	4.93	4	1.224	3.76	1.0638
69	*Penicillium* sp. 2	Trichocomaceae	0	3.55	0	5.12	0.693
70	*Penicillium* sp. 3	Trichocomaceae	0	7.56	0	4.81	1.571
71	*Periconia hispidula*	Incertae sedis	4.13	3.54	1.167	4.85	0.729
72	*Phoma glomerata*	Incertae sedis	0	3.39	0	4.66	0.727
73	*Phoma* sp.	Incertae sedis	4.96	4.54	1.092	5.75	0.789
74	*Phoma* sp. 1	Incertae sedis	0	4.54	0	4.95	0.917
75	*Rhizopus* sp.	Mucoraceae	0	3.13	0	5.14	0.609
76	*Rhizopus stolonifer*	Mucoraceae	0	5.33	0	6.15	0.867
77	*Scytalidium lignicola*	Incertae sedis	2.96	2.56	1.157	3.22	0.795
78	*Scytalidium thermophilum*	Incertae sedis	0	7.86	0	5.96	1.319
79	Sterile fungi 1	-	0	4.74	0	4.75	0.998
80	Sterile fungi 2	-	0	5.76	0	3.68	1.565
81	Sterile fungi 3	-	0	3.76	0	3.15	1.193
82	Sterile fungi 4	-	0	2.87	0	4.68	0.613
83	Sterile fungi 5	-	0	2.34	0	2.68	0.873
84	Sterile fungi 6	-	0	5.46	0	4.93	1.108
85	Sterile fungi 7	-	0	3.03	0	2.23	1.359
86	Sterile fungi 8	-	0	5.13	0	3.21	1.644
87	Sterile fungi 9	-	0	3.34	0	4.74	0.705
88	Sterile fungi 10	-	0	5.16	0	4.65	1.109
89	*Trichoderma aureoviride*	Hypocreaceae	0	6.83	0	5.37	1.272
90	*Trichoderma polysporum*	Hypocreaceae	3.33	2.93	1.137	4.25	0.689
91	*Trichoderma pseudokoningii*	Hypocreaceae	0	6.56	0	6.87	0.955
92	*Trichoderma reesi*	Hypocreaceae	0	3.21	0	5.42	0.592
93	*Trichoderma* sp.	Hypocreaceae	0	4.65	0	5.18	0.898
94	*Trichoderma* sp. 1	Hypocreaceae	0	3.36	0	3.43	0.979
95	*Trichoderma strctipilis*	Hypocreaceae	0	6.26	0	6.17	0.932
96	*Trichoderma viride*	Hypocreaceae	0	7.64	0	4.21	1.814
97	*Tritriachium dependens*	Trichocomaceae	0	2.43	0	3.64	0.668
98	*Verticillum* sp.	Plectorsphaerellaceae	0	6.03	0	6.17	0.977
99	*Verticillum terrestre*	Plectorsphaerellaceae	0	5.06	0	6.39	0.791

Note: REA= Relative enzyme activity index (values more than 0 showed positive amylase enzymatic activity), HZ= Hydrolysis zone, CD= Colony diameter, CDP= Colony diameter on potato dextrose agar, GS/II= Growth simulation/inhibition index.

Growth simulation (G.S.) /inhibition index (I.I.) was computed as the colony diameter on starch hydrolysis agar/colony diameter on control agar ratio (Verma & Verma 2016a). The index value <1, represented substrate inhibited fungal growth, while the index value >1, exhibited substrate rendered growth stimulation. Range of growth stimulation (GS) 2.279–1.025. Test fungi give highest growth stimulation on an enzymatic medium such as Non sporolating hypomyctes (2.279) followed by *Oidiodendron mais* (2.121), *Cephalosporium indicum* (2.079), *Absidia fuca* (1.976), *Trichoderma viride* (1.814), *Nigrospora oryzae* (1.724) Range of inhibition index (II) 2.279–1.025. Fungi give highest I.I. *Aspergillus fumigates* (0.376), *Aspergillus parasiticus* (0.498), *Penicillium citreonigrum* (0.509), *Aspegillus janus* (0.555), *Memmoniella echinata* (0.556), *Aspergillus* sp. 2 (0.591) and *Trichoderma reesi* (0.592). However, hydrolysis zone diameters were equal to colony diameter in case *Penicillium nigricans* (0).

Furthermore, the colony growth of 41 tested fungi on starch hydrolysis agar was found to be greater as compared to control, slightly greater in 13 test fungi, growth was slightly inhibited in 37 test fungi and colony diameter was reduced in 7 fungi (Fig. 1). None of the isolates were found to be resistant for starch hydrolysis agar (Table 1).

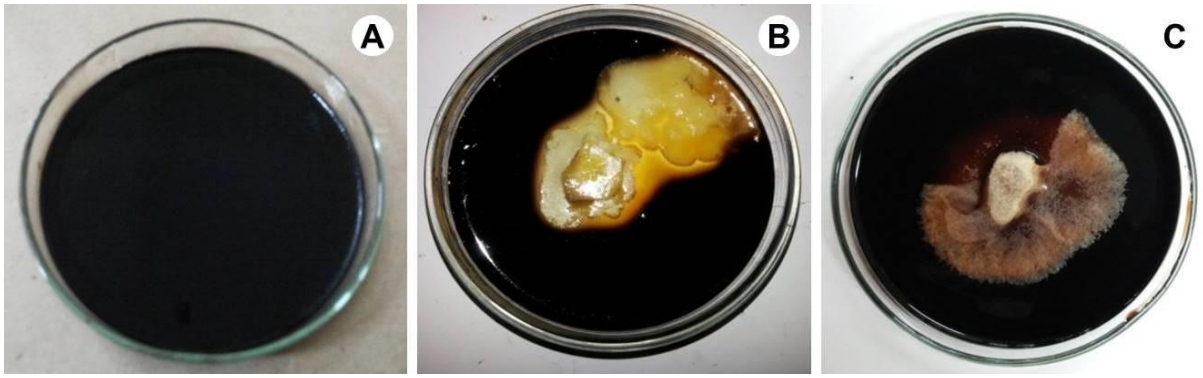

Figure 1. Amylase enzymatic activity: **A**, Control plate; **B**, Zone of hydrolysis activity; **C**, Negative result of amylase activity.

Isolated 29 genera were mainly belonging to 10 families. Maximum 44 fungi were isolated from Trichocomaceae family and one fungus from Chaetomiaceae and Myxotrichaceae family. Isolated fungi of Chaetomiaceae, Mucoraceae, Mycosphaerelleaceae, Myxotrichaceae and Nectriaceae family were unable to produced enzyme. Maximum numbers of fungi were produced enzyme belonging to Trichocomaceae family, followed by Incertae sedis (Fig. 2).

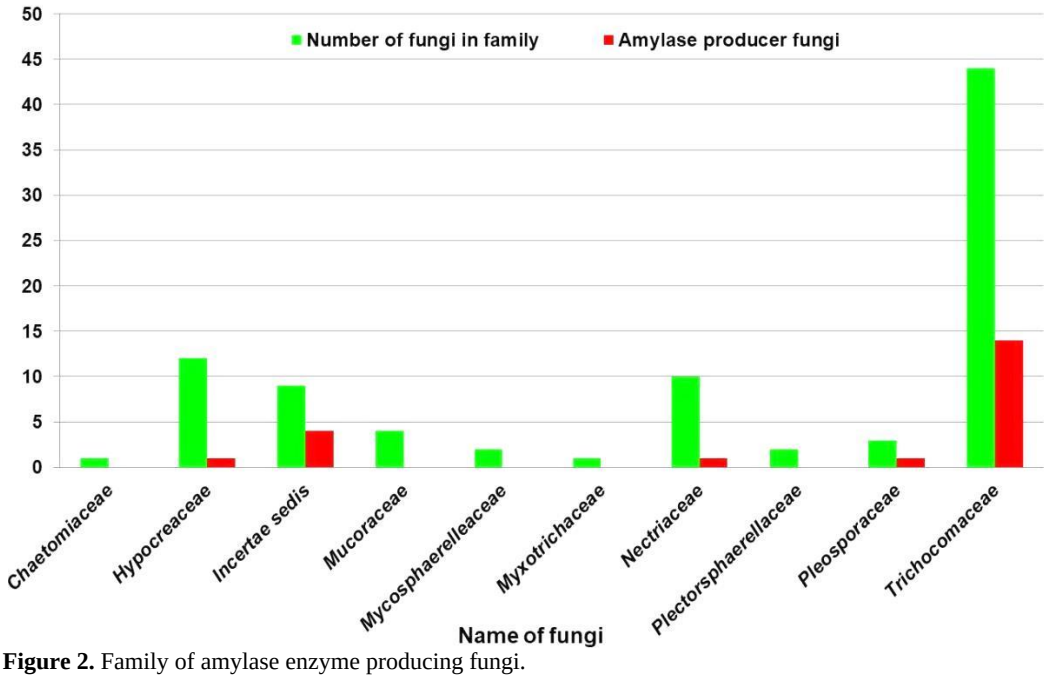

Figure 2. Family of amylase enzyme producing fungi.

DISCUSSION

Screening of fungi on the basis of amylase activity

The saprophytic fungi represent the largest proportion of soil fungi and they perform a crucial role in the decomposition of plant structural polymers, such as cellulose, hemicelluloses, lignin and starch, thus contributing to the maintenance of global carbon cycle. The distribution of these organisms is influenced by the abundance and nature of the organic content of the soil, as well as by other soil texture (Waksman 1944). Fungi are known agents of decomposition of organic matter, by producing extracellular enzymes (Reese & Levinson 1952, Lynd *et al*. 2002, Chi *et al*. 2007, Gupta *et al*. 2008). Due to wide diversity, fungi have been recognized as a source of the new enzyme with useful and /or novel characteristics.

In the present findings maximum amylase enzyme was produced by *Penicillium* sp. 1 and Yamasaki *et al*. (1977), Ertan *et al*. (2006), Ogbonna *et al*. (2014), Verma *et al*. (2015b) were also observed similar results but the source of isolation of fungi was different. *Aspergillus* sp. was produced enzyme in medium this finding was

supported by Razzaque & Ueda (1978), Abe *et al*. (1988), Kunamneni *et al*. (2005), Ragunathan & Swaminathan (2005), Bakri *et al*. (2009), Toye (2009), Ominyi *et al*. (2013), Ogbonna *et al*. (2014), Verma *et al*. (2015b) but source of isolation of fungi was different. *Trichoderma polysporum* was produced the enzyme in medium this finding was supported by Mishra & Maheshwari (1996), but source of isolation of fungi was different. *Fusarium chlamydosporum* was produced the enzyme in medium these finding was supported by Pathak *et al*. (2014) but the source of isolation of fungi was different. *Scytalidium lignicola, Memmoniella echinata* and *Periconia hispidula* were isolated from leaf litter of Eucalyptus (Dorai 1988) *Phoma* sp. and *Alternaria alternata* was produced enzyme in medium this finding was supported by Masumi *et al*. (2014) but source of isolation of fungi was different.

Amylase activity of *Bacillus cereus* isolated from a coal mine was studied by Bhatt *et al*. (2015). A similar study was also done by Parida *et al*. (2014) in iron ore mine bacterial isolates. Microbial enzymes like amylase and cellulase are extracellular enzymes that play a vital role in the nutrient cycling of the soil (Tabatabai 1994). However, soil microbial enzymes are considered to be indicative measures of soil fertility and bioremediation activities (Margesin *et al*. 2000). In this investigation, it was found that mining soils harbor fewer microorganisms with less diversity. Therefore, microbial function in the mining soil could be attributable to low microbial diversity and confined to a specific group of microorganisms (Khan & Joergensen 2009, Parida *et al*. 2014) (Table 1).

In the present finding *Acremonium strictum, Penicillium oxalicum, Aspergillus niger, Rhizopus* sp. and *Aspergillus flavus* give negative result, But Yamasaki *et al*. (1977), Takahashi *et al*. (1978), Purushotham *et al*. (1996), Jain *et al*. (2012) and Sundar *et al*. (2012) observed *Acremonium* sp., *Penicillium oxalicum*, *Aspergillus niger* and *Aspergillus flavus* produced amylase enzyme. Hence, there is an increasing worldwide interest in the screening of new microorganisms producing amylases suitable for industrial applications (Burhan *et al*. 2003).

Studies on fungal amylases especially in developing countries have concentrated mainly on ubiquitous fungi like, *Aspergillus, Penicillium, Alternaria* etc. probably because of their ubiquitous nature and nonfastidious nutritional requirements of these organisms (Azevedo 2000, Suganthi *et al*. 2011, Sidkey *et al*. 2011, Khan & Yadav 2011).

Fungi give negative results in amylase medium

Out of 99 fungi, 78 fungi were unable to produced amylase enzyme (Table 1). Thus the lack of a positive result could mean that either the enzyme is not produced, or that it is produced intracellular and not released from the mycelium, or that it is produced and released, but the medium inhibits its detection. The relationship between the ability to grow on a particular test medium and to produce the corresponding enzyme to digest the substrate incorporated into the medium is not well correlated (Egger 1986, Pointing 1999, Abdel-Raheem & Shearer 2002). This may be because fungus uses other material from the medium and another carbon source rather than substrates added (Yuen *et al.* 1998, Abdel-Raheem & Shearer 2002, Verma & Verma 2016a, Verma & Verma 2016b).

CONCLUSION

Penicillium sp. 1 showed the highest amylase activity, which may be applied in mine overburden soil along with amendment of organic matter to improve fertility of overburden.

ACKNOWLEDGEMENT

Authors are thankful to Director, Tropical Forest Research Institute, Jabalpur for providing laboratory facilities.

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