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Research article

Heavy metal contaminated acidic soil: A source of useful fungi

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Abstract: The incidence of fungi endowed with exploitable potential in acidic laterite soil was studied. The botanic garden located nearby to Chandaka forest of Odisha was considered the site for collection of soil sample. Among the 16 fungal isolate found in the soil4 belonged to *Aspergillus* sp., 1 belonged to *Cladosporium* sp., 6 belonged to *Penicillium* sp. while 5 were Sterile mycelium. All the sixteen fungi were characterized morphologically and screened for extracellular production of enzymes, organic acids and antagonistic behaviour. It is observed that most of the fungi are amylase, lipase, L-asparaginase producer while few of them werepositive for xylanase production. Antagonistic behaviour of sterile mycelium 7 exhibited its wide spectrum activity against fungi tested. It is evident that soil having acidic pH and contaminated with heavy metal is inhabited with useful fungi.

Keywords: Soil fungi - Heavy metal contamination - Antagonism.

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INTRODUCTION

Soil is a conglomerate system which harbours almost all major groups of fungi. Fungi dominate in soil in terms of biomass but vary in number and kind with increasing depth of soil (Nagamani *et al.* 2006). They occur in soil either in mycelial state or reproductive stage. Hyphae, hyphal fragments and sporulating fungi are also present in soil (Nagamani *et al.* 2006). They occupy a variety of ecological habitats because of their immense adaptability capacity; hence can be isolated even from unusual habitats such as salterns, peat soil, petroleum polluted soils, inner tissues of plants and animals (Rajagopal *et al.* 2010). Fungi are relatively important than bacteria in organic soils with low pH. They are known to be primary decomposers and recycle stored energy and nutrients of the organic matter.Soil fungi are most studied group of fungi, and soil genera such as *Acremonium*, *Aspergillus*, *Fusarium* and *Penicillium* can alsosynthesize a wide range of useful bioactive compounds. More than 30% of isolated metabolites from fungi are found from *Aspergillus* and *Penicillium* (Bérdy 2005). These microorganisms mediate soil processes such as nutrient mobilization and mineralization, soil organic matter decomposition, phosphate solubilization and nitrogen fixation, nitrification, denitrification and sulphur reduction (Khan *et al.* 2007).

The industrial revolution and anthropogenic activities alleviated the metal concentration in soil (Yan-de *et al.* 2007, Oves *et al.* 2012). Presence of excessive load of heavy metals in soil due to deposition of heavy metals leads to heavy metal stress which results in pollution of soil. The excessive metals in soil also affect soil properties and fertility making unsuitable for agricultural activities. Metal accumulation also results in reduced microbial population (Wani & Khan 2013). However certain micro-organisms can survive in this type of abiotic stress conditions. There are reports that metal tolerant micro-organisms have useful activity which help in growth promotion and have positive effects onplants (Selvakumar *et al.* 2012, Oves *et al.* 2013, Sahu *et al.* 2014, Vyas & Gupta 2014). These microbes are also good source of industrial enzymes, organic acids and antibiotics (Khan *et al.* 2009, Bello *et al.* 2016).

The present study was aimed to analyse the incidence of fungi in the soil having heavy metals and acidic pH. Further, the characterization for their extracellular activity like enzyme production and mineral solubilisation was the prime objective in order to search for the exploitable potential of these fungi.

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MATERIALS AND METHODS

The red laterite soil was collected from the botanic garden of Regional Plant Resource Centre, Bhubaneswar and treated for soil analysis (Jackson 1958). Serial dilution, plate culture and slide culture techniques (Mehrotra & Aneja 1990) was used for the isolation of fungi and their morphologic characterization and tentative identification. Colony characteristics of all the organisms were noted which includes the front and back appearance of the organism on medium plate, growth pattern, texture, elevation and margin of the fungal colony after 5 days of incubation at 25ºC. All the fungal cultures were characterized for their extracellular enzymatic activity, phosphate solubilisation capacity, IAA production and antagonistic activity (Sahoo *et al.* 2014, Rajoriya *et al.* 2014).

RESULTS AND DISCUSSION

In the present study, soil used for the analysis of incidence of fungi was highly acidic (pH 5.6–6.0). Mineral analysis of the soil was confirmed the presence of Fe (32 $mg.kg^{-1}$), Mn (11 $mg.kg^{-1}$), Cu (1 $mg.kg^{-1}$) and Zinc (0.3 $mg.kg^{-1}$) along with N, P, K in the ratio of 9:1:4. It was possible to isolate almost 16 numbers of fungi characterized morphologically, identified at the generic level and categorised into *Aspergillus, Penicillium, Cladosporium* and Sterile mycelium. All the 16 organisms were different in their colony morphology and vary from each other in front and back appearance on medium plate, growth, colony characteristics such as margin, texture and elevation (Table 1).

Table 1. Colony characteristics of fungi isolated from acidic laterite soil.

S.N.	Organism	Front Colour	Reversecolour	Growth	Form	Margin	Texture	Elevation
1	*Aspergillus* sp. 11	Brown To Pale Yellow	Dark Brown	++	Irregular	Entire	Velvety	Flat
2	*Aspergillus* sp. 12	Black	Pale Yellow	++++	Irregular	Filliform	Powdery	Raised
3	*Aspergillus* sp. 13	Dark Green	Pale White	++++	Irregular	Filliform	Powdery	Raised
4	*Aspergillus* sp. 14	Black	Pale Yellow	++++	Irregular	Filliform	Powdery	Flat
5	*Cladosporium* sp.	Black	Black With White Margin	++	Irregular	Entire	Velvety	Flat
6	*Penicillium* sp. 5	Grey	Black	++	Irregular	Undulate	Velvety	Raised
7	*Penicillium* sp. 6	Olive Green	Pale Yellow	++++	Irregular	Filliform	Velvety	Flat
8	*Penicillium* sp. 7	Grey	Pale Orange	+++	Irregular	Undulate	Slight Cottony	Flat
9	*Penicillium* sp. 8	Light Sap Green	Pale White	++++	Irregular	Undulate	Powdery With Slight Cottony	Flat
10	*Penicillium* sp. 9	Olive Green	Pale White	+++	Irregular	Undulate	Slight Cottony	Raised
11	*Penicillium* sp. 10	Brown To Pale Yellow	Dark Brown	+++	Irregular	Undulate	Slight Cottony	Raised
12	*Sterile mycelium* sp. 3	Cream	Cream	++	Irregular	Undulate	Slightly Cottony	Flat
13	*Sterile mycelium* sp. 4	Black With White Border	Brown	+	Irregular	Entire	Slighty Cottony	Flat
14	*Sterile mycelium* sp. 5	Blackish With Whitish Cottony	Black To Brown	++++	Filamentous	Filliform	Cottony	Raised
15	*Sterile mycelium* sp. 6	Olive Green To Yellow	Pale Yellow	++++	Circular	Entire	Slight Cottony	Flat
16	*Sterile mycelium* sp. 7	White	White	++++	Circular	Filliform	Cottony	Raised

Note: (+++++), Maximum growth; (++++), Good growth; (+++), Moderate growth; (++), Less growth; (+), Least growth.

Occurrence of various fungi and their mineral solubilising potential in mine environment has been documented very well (Gupta *et al.* 2007). It is important to note that most of the fungi isolated from the heavy metal contaminated soil are found to be phosphate solubilising nature in the present study.Most of them were L-asparaginase, protease and lipase producers and few among them were producing xylanase, amylase and cellulase when screened through plate culture in specific medium (Table 2). Co-inoculation plate test performed for the evaluation of antagonistic behaviour of these fungi and exhibited the antifungal potential of sterile mycelium. This fungus had inhibitory activity towards the growth of 5 *Aspergillus* and 2 *Penicillium* sp. among the 16 test fungi used for the experiment (Table 3).

Table 2. Extracellular enzyme, organic acid production and phosphate solubilisation activity of fungal isolates.

Organism	Amylase	Cellulase	Iaa	L- Asparginase	Lipase	Organic Acid	Phosphate Solubilisation	Protease	Xylanase
Aspergillus sp. 11	+++	++	-	-	-	++++	+	++	+
Aspergillus sp. 12	+	-	-	++++	++	+	-	+++	-
Aspergillus sp. 13	-	++++	-	++	-	+++	-	-	+++
Aspergillus sp. 14	-	-	-	+	++	-	-	+++	-
Cladosporium sp.	+	-	-	+	+++	++	-	-	-
Penicillium sp. 5	-	-	-	+	+	-	-	-	-
Penicillium sp. 6	-	-	-	+	+	++	+	-	-
Penicillium sp. 7	-	-	-	+	+++	-	+	++	-
Penicillium sp. 8	-	-	-	+	++	-	+	-	-
Penicillium sp. 9	+	-	-	-	-	-	+	+	-
Penicillium sp. 10	+	-	-	+	++	+	+	++	-
Sterile mycelium sp. 3	-	-	-	-	-	-	++	+++	-
Sterile mycelium sp. 4	-	+++	-	++	-	-	++	+	-
Sterile mycelium sp. 5	++	-	-	-	-	++++	++	++	-
Sterile mycelium sp. 6	-	-	-	++	++	-	++	-	-
Sterile mycelium sp. 7	+	+++	-	++	-	+++	+++	-	-

Note: (+++++), Highest activity; (++++), High activity; (+++), Good activity; (++), Medium activity; (+), Low activity; (-), No activity.

Table 3. Antagonistic properties of fungal isolates.

Soil fungi	*A1*	*A 2*	*A 3*	*A 4*	*A 5*	*A 6*	*A 7*	*A 8*	*A 9*	*A 10*	*P 1*	*P 2*	*P 3*	*P 4*	*S 1*	*S 2*
A 11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A 12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A 13	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-
A 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***Cl* sp.**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P 7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S 3													+			
S 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S 6	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
S 7	+	-	-	+	-	+	-	-	+	+	-	+	+	-	-	-

Note: +, Inhibitory activity; **-,** No effect on growth.
(*A 1*, *Aspergillus* sp. 1; *A 2*, *Aspergillus* sp. 2; *A 3*, *Aspergillus* sp. 3; *A 4*, *Aspergillus* sp. 4; *A 5*, *Aspergillus* sp. 5; *A 6*, *Aspergillus* sp. 6; *A 7*, *Aspergillus* sp. 7; *A 8*, *Aspergillus* sp. 8; *A 9*, *Aspergillus* sp. 9; *A 10*, *Aspergillus* sp. 10; *A 11*, *Aspergillus* sp. 11; *A 12*, *Aspergillus* sp. 12; *A 13*, *Aspergillus* sp. 13; *A 14*, *Aspergillus* sp. 14; *Cl* sp., *Cladosporium* sp.; *P 1*, *Penicillium* sp. 1; *P 2*, *Pencilllium* sp. 2; *P 3*, *Penicilllium* sp. 3; *P 4*, *Penicillium* sp. 4; *P 5*, *Penicillium* sp. 5; *P 6*, *Penicillium* sp. 6; *P 7*, *Penicillium* sp. 7; *P 8*, *Penicillium* sp. 8; *P 9*, *Penicillium* sp. 9; *P 10*, *Penicillium* sp. 10; *S 1*, *Sterile mycelium* 1; *S 2*, *Sterile mycellium* 2; *S 3*, *Sterile mycellium* 3; *S 4*, *Sterile mycellium* 4; *S 5*, *Sterile mycellium* 5; *S 6*, *Sterile mycellium* 6; *S 7*, *Sterile mycellium* 7).

It is confirmed that fungi present in the soil of acidic nature contaminated with heavy metal content are endowed with some useful and exploitable potential of some industrial product like enzymes, organic acids etc. Observation on their phosphate solubilising potential also makes them agriculturally important besides their industrial potential. The negative antagonist behaviour for other fungi is an aided advantage to make these fungi agriculturally more useful as bio fertiliser development. Hence, it is inferred that this acidic laterite soil is endowed with a good number of fungal strains having exploitable potential. Further systematic research on enzyme extraction, purification and its utility, as well as their impact on growth and development of agriculturally important crops and other plants of economic importance is required to reach any conclusion.

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