RESEARCH ARTICLE

Fusarium oxysporum as a potential fungus for bioremediation

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ABSTRACT

Fungi occupy many different ecological niches. Fungi are well known to tolerate and detoxify metals by several mechanisms including extra- and intracellular precipitation, and active uptake. In the present paper soil fungi is isolated from forest soil samples from different locations of SGNP. *Fusarium oxysporum* was examined for its pH tolerance, Metal tolerance and biosorption capacity to Zn and Pb metals. The effect of the metal pollutants on the fungi and response of the fungi was analysed using ICP AES. The changes in the mycelial characteristics were observed. *Fusarium oxysporum* proves to be a potential candidate for bioremediation

Key words: *Fusarium oxysporum*, biosorption

INTRODUCTION

Metals may be present in soils as free metal ions, complexes with organic matter or they may be chemically precipitated into insoluble compound such as oxalates, carbonates and hydroxides. The degree of toxicity of the metal to the organisms depends on its relative availability (solubility) within the soil solution. This availability is dependent upon edaphic factors such as pH, organic matter and clay content. Soil microfungi are able to tolerate concentrations of various metals and less sensitive at higher pH levels. Fungi are able to restrict the entry of toxic metal species into the cells by extracellular metal sequestration, binding to the cell wall, reducing its uptake by intercellular chelation (Berthlin *et al* 1995; Gadd 2007).

Fungi which are a major and often dominant component of the microbiota of soil are important as decomposer organism, animal and plant symbionts and pathogens and they play an important role in biogeochemical cycle of elements. Certain fungal processes solubilise metal from minerals and bound locations thereby increasing metal bioavailability, whereas other fungal processes immobilize and reduce their bioavailability (Fomina *et al* 2005). Naturally fungi have a variety of extracellular proteins, organic acids and other metabolites. Fungi can adapt in any ecosystem and any environmental conditions.

Rapid urbanization and industrialization has led to increased occurrence of heavy metals in the environment. The resultant degradation and contamination of ecosystem become a major threat to all living organism globally open water and aquatic ecosystems are contaminated with several heavy metals to human activities. Bioremediation is considered alternative processing methods for removing the heavy metal ions from polluted areas(Hardman et al 1993).

The present paper focuses on the role of *Fusarium oxysporium* as potential fungi for bioremediation. The fungi was investigated for the metal tolerance capacity, biosorption capacity and the changes in mycelia characteristics.

MATERIALS AND METHODS

Soils samples from different locations in the forest of Sanjay Gandhi National Park were collected.

Soil sampling

Soil samples were collected by taking composite samples up to a depth of 10cm, after scraping off 5cm of surface soil with a sterile trowel. Soil samples were collected in fresh polyethene bags. The soils were brought to the laboratory for further study. Soil was dried and then used for isolating soil fungi.

Isolation of fungi

Soil fungi were cultured on PDA, soil agar and Czapek dox .The Petri plates were incubated at 28^0 C for 7 days. The fungi were isolated and maintained as pure cultures. They were examined under light microscope and were identified using standard manuals. Identification of soil fungi was done on the basis of microscopic and colony characteristics and the basic identification keys were used. Digital Photographs of fungi were taken using MIPS.

Isolated soil fungi were *Absidia, Acremonium sp, Alternaria sp, Aspergillus sp, Cladosporium sp, Cylindrocarpon, Fusarium oxysporum, Fusarium solani, Geotrichum sp, Gliocladium sp, Mucor, Paecilomyces, Penicillium sp, Rhizopus, Trichoderma ,Verticillium.* It was observed that *Fusarium oxysporum* was found in most of the collected soil samples. Of the isolated fungi *Fusarium oxysporum* was selected for further experiments.

pH tolerance

F.oxysporum was cultured on PDA with varying pH to check its tolerance .pH was adjusted in the range of 5 to 14 using Na_2CO_3.Cultures were grown for 15 days. Growth of the fungus in pH 5 to pH 14 was expressed in terms of biomass weight. It was observed that *F.oxysporum* is growing in pH 5 -11. (Table 1)

Metal tolerance and biosorption capacity

Fusarium oxysporum cultures were grown on Czapek dox broth (CZB) amended with metal solutions (Zn and Pb) of varying concentrations (200, 400, 600, and 800 ppm). Control flask containing Czapek dox broth inoculated with culture was kept as reference. The flasks were kept at 28+-2 c for 15 days on shaker. The biomass was harvested and filtered through Whatman no. 42 filter paper after 15 days of growth. The weight of biomass in each concentration was determined.

The harvested mat was oven dried until a constant weight was reached. After the dry weight was measured 1 gm of dried mat was digested in 10 ml of concentrated nitric acid. After appropriate dilutions with double distilled water, metal ions contents of the solutions and culture filtrates were analyzed for the presence of Zn and Pb using Inductive coupled plasma (ICP AES) at SAIF IIT Mumbai.

Determination of metal tolerance index

The metal tolerance index (MTI) was determined to assess the ability of the fungi to grow in the presence of a given concentration of Pb and Zn according to the following equation:

$$\text{MTI (\%)} = \frac{\text{DW of treated culture}}{\text{DW of control culture}} \times 100$$

Where DW is the dry weight of the culture

Determination of biosorption capacity

The biosorption capacity of Zn and Pb was calculated using the formula

$$Q = \frac{(ci - cf)}{m} V$$

Where Q = milligram of metal ions uptake per gram biomass (mg/g)

C_i = initial concentration of metallic ions (mg/L)

C_f = final concentration of metallic ions (mg/L)

m = dried mass of biosorbent in reaction mixture (gm)

V = volume of reaction mixture (ml)

Change in mycelial characteristics of *Fusarium oxysporum*

The mycelia from the different flasks of varying concentrations were harvested after 15 days growth. Changes in the morphological characteristics of the mycelium were noted using light microscope.

RESULTS AND DISCUSSION

25 Soil samples were collected from different locations of Sanjay Gandhi National Park forest. Around 35 fungi were isolated in culture. Of all the isolated fungi, *Fusarium oxysporum* (**Fig. a**) was observed in all the soil samples. Tolerance of this fungus ranged from pH 5 to pH 11. Growth in the different pH was measured as biomass weight (**Table 1**). This shows the versatility of *Fusarium oxysporum* to adapt to acidic and basic pH conditions.

The culture was subjected to varying concentrations of Zn and Pb to check the metal tolerance index. It was observed that metal tolerance index for Zn in 200 ppm was 51% , 400ppm 28%,600 ppm 7% and in 800 ppm 3.7 % showing decrease in metal tolerance index with increase in concentration.(**Table 2**)

In Pb amended media it was observed 200 ppm was 53% , 400ppm 25%,600 ppm 11% and in 800 ppm 1% showing decrease in metal tolerance index with increase in concentration.(**Table 3**).

Biosorption capacity of the Zn and Pb in the different concentrations 200, 400, 600 and 800 was 8.8, 7.4, 3.2 and 7.8 for Zn. Similarly for Pb it was 3.0, 3.8, 6.0 and 11.6 respectively.

Certain fungal processes solubilise metals from minerals and bound locations, thereby increasing metal bioavailability, whereas other fungal processes immobilize metals and reduce their bioavailability. Flexible mycelial growth strategies and the ability to produce and exude organic acids, protons, and other metabolites make fungi important biological weathering agents of natural rock, minerals, and building materials.

Table 1: pH tolerance of *F.oxysporum*

pH	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Biomass (mg)	8.5	9.2	8.8	6.2	4.1	2.8	1.2	00	00	00

Table 2: Metal tolerance index of *F.oxysporum* Zn amended media

Concentration(ppm)	**Wet weight(mg)**	**Dry weight(mg)**	**MTI (%)**
200	5.9	3.8	51
400	4.61	2.1	28
600	1.734	0.583	7
800	0.938	0.278	3.7
Control	15.26	7.45	--

Table 3: Metal tolerance index of *F.oxysporum* Pb amended media

Concentration(ppm)	**Wet weight(mg)**	**Dry weight(mg)**	**MTI (%)**
200	6.24	3.98	53
400	4.12	1.92	25
600	1.24	0.893	11
800	0.544	0.123	1
Control	15.26	7.45	--

Table 4: Biosorption capacity of *F.oxysporum* in metal amended media

Concentration	**200 ppm**	**400 ppm**	**600 ppm**	**800 ppm**
Zn amended media (mg/g)	8.8	7.4	3.2	7.8
Pb amended media (mg/g)	3.0	3.8	6.0	11.6

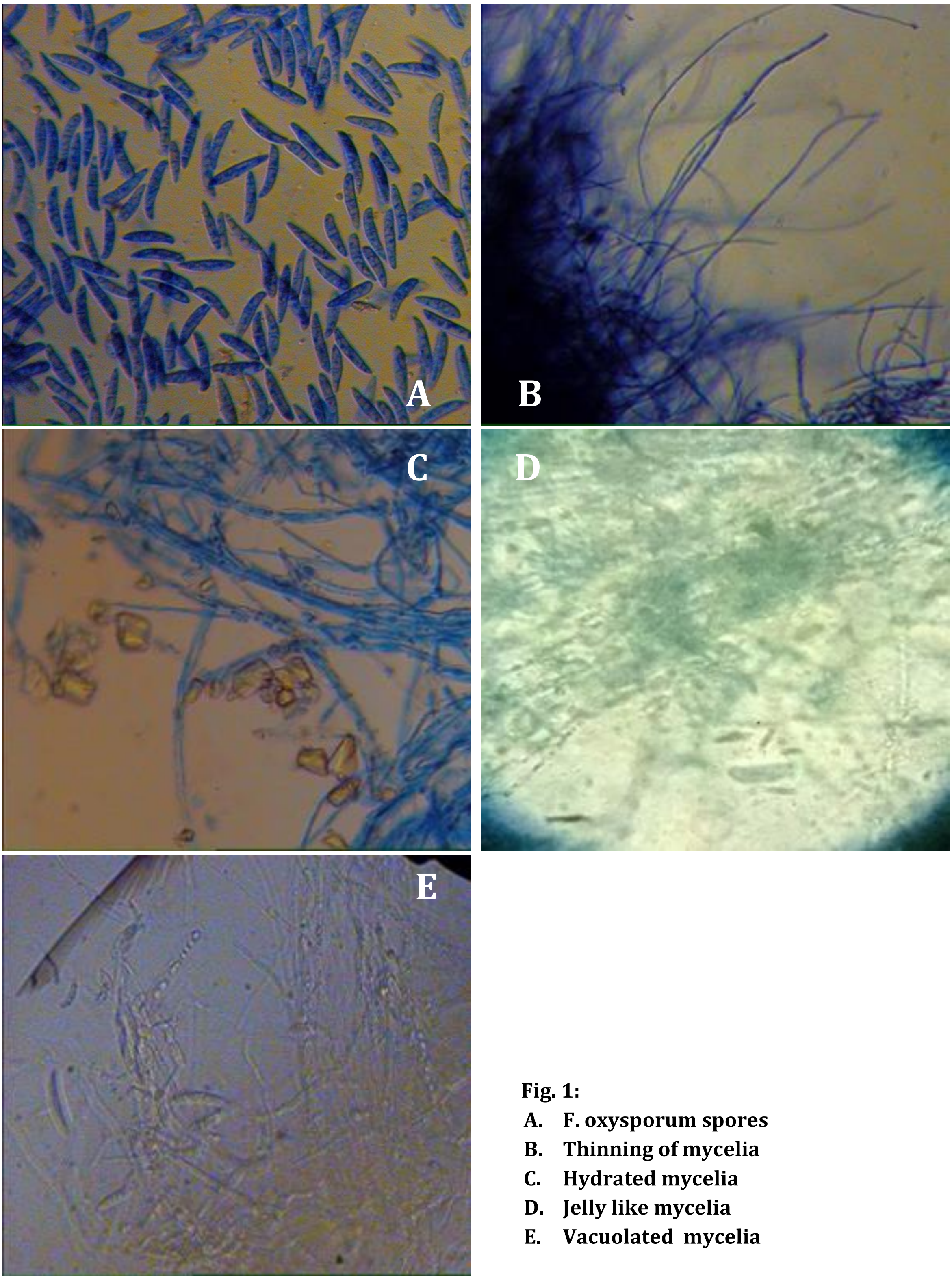

Fig. 1:

A. **F. oxysporum spores**
B. **Thinning of mycelia**
C. **Hydrated mycelia**
D. **Jelly like mycelia**
E. **Vacuolated mycelia**

In *F.oxysporum* the following changes in the mycelia were observed. Mycelia Straight branched length 12µ m and 4.5 µm in control culture, whereas the growth with metal media (200 and 400ppm) it has shown the changes in mycelia thin dense soft vacuolated, mucilaginous 12µm length and 4.5µm width spore size 54µm length and 4.5µm width. Formation of mycelia covered by a thick hydrated mucilaginous sheath leading to formation of jelly like mass which provide a micro environment for chemical reactions, crystal depositions and growth.(**Figs b, c, d, e**,) Thereby leading to reduced mobilization of metals.

CONCLUSION

As mineral component contain considerable quantities of metals as well as other elements which are biologically unavailable, the influence of such processes on metal mobility are of economic and environmental significance and may be important in the treatment of contaminated soil. From the above observations *F.oxysporum* proves to be a potential candidate for bioremediation.

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