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Resistant Fungal Biodiversity of Electroplating Effluent and Their Metal Tolerance Index

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1. Introduction

Discharge of heavy metals in the aquatic system has become a global phenomenon due to their carcinogenic and mutagenic nature (Mahiva *et al*., 2008). electroplating industry in Pakistan is contributing its major part in deteriorating the country environment at massive scale with the accumulation of heavy metals in aqueous environment. The chemically polluted water has seriously damaged the ecology of surface and ground water, which eventually impart serious consequences on agriculture due to contamination of crops grown in a polluted area .

The introduction of heavy metal compounds into the environment generally induces morphological and physiological changes in the microbial communities (Vadkertiova and Slavikova, 2006), hence exerting a selective pressure on the microbiota (Verma et al., 2001). Strains isolated from contaminated sites have an excellent ability of removing significant quantities of metals from effluents (Malik, 2004). Conventional processes such as chemical precipitation; ion exchange and reverse osmosis are uneconomical and inefficient for treating effluents (Gupta et al., 2000; Pagnanelli et al., 2000; Gavrileca, 2004; Malik, 2004).Biosorption, using bacteria, fungi, yeast and algae, is regarded as a cost-effective biotechnology for the treatment of wastewaters containing heavy metals. Among the promising biosorbents for heavy metal removal which have been researched during the past decades, fungi has received increasing attention due to their higher ability to remove high concentrations of heavy metals than yeast, bacteria and and algae ((Gavrilesca, 2004; Baldrian, 2003; Zafar et al., 2007).They can adapt and grow under high metal concentrations (Anand et al., 2006). They offer the advantage of having cell wall material which shows excellent metal-binding properties (Gupta et al., 2000). Fungi are known to tolerate metals by several mechanisms including valence transformation, extra and intracellular precipitation and active uptake (Malik, 2004). Considering the above mechanisms of metal resistance in fungi, we have studied filamentous fungi isolated from electroplating industrial effluent, a polluted environment to assess their metal tolerance and metal removal potential from aqueous solution. High affinity, rapid rate of metal uptake and maximum loading capacity are important factors for the selection of a biosorbent. Therefore, there is an

increased interest in the identification of some new and better biosorbents that show promising uptake of metallic ions (Akhtara *et al*., 2007). It is expected that screening of metal tolerant fungi may provide strains with improved metal accumulation. The introduction of heavy metal compounds into the environment generally induces morphological and physiological changes in the microbial communities.The aim of the present study was to study Cu resistant fungal biodiversity and their metal tolerance index from electroplating effluent. The study was also aimed to analyse functional group responsible for Cu removal. There is huge literature on the biosorption of heavy metals from electroplating effluent but there was no attention on the *Gliocladium* sp. as potential biosorbent. In this present work *Gliocladium viride* was selected after screening of 50 fungal isolates. Potential fungal strain was selected on its comparative biosorption capacity.

To the best of our knowledge this work is the first report on biosorption of Cu by *Gliocladium viride*. This non-pathogenic fungus belongs to Ascomycota division. It is well known for the production of lytic enzymes (cellulases and chitinases) and antibiotics. It also acts as a biocontrol agent against plant pathogens (Druzhinina *et al*., 2005). The effluent samples were characterized for its physicochemical parameters.A total of fifty filamentous fungal strains representing five genera; *Gliocladium, Penicillium, Aspergillus, Rhizopus* and *Mucor* species, were isolated and screened for their Cu removal efficiencies from electroplating tanning effluent. The experimental results showed that *Gliocladium* sp. was the best Cu resistant fungus among all fungal species isolated from the effluent. These strains were exposed to Cu metal ions up to 3mM to study tolerance of isolated fungal.The degree of tolerance was measured from the growth rate in the presence of Cu. Whole mycelium and cell wall component were analyzed for Cu biosorption. Cell wall component was found to be responsible for Cu biosorption. Amino groups were found to be abundant in the cell wall of *Gliocladium viride* ZIC_{2063} as determined by infrared spectroscopy. These examinations indicated the involvement of amines in metal uptake.

2. Matherial and methods

2.1 Isolation and scerening of fungi for cutolerance

The PDA (Potato Dextrose Agar) medium was used for culturing fungi from tanning effluent. One ml of serially diluted (1000-fold) effluent was spread on the PDA agar plate. The inoculated plates were incubated at 30 ^{0}C for 72 h. The morphologically distinct colonies were selected and screened for their Cutolerance. A small piece of mycelium (3 mm^2) was inoculated on PDA plates supplemented with 3mM copper sulphate.The plates were incubated for seven days at 25 ^{o}C. The control (without metal solution) was also run in parallel. Reduction of radial growth rate was used as an index for metal tolerance. To compare the heavy metal tolerance of each isolate, a parallel index of tolerance (T.I) was calculated as a percentage value from the ratio:

$$\text{T. I.} = \frac{\text{Radial growth rate in metal treatment}}{\text{Radial growth rate in control}}$$

The isolates exhibiting better growth after incubation were considered as tolerant to the metal.

2.2 Biosorption studies

All fungal strains were further screened to check their Cu removal efficiencies. Potential of fungal strains for Cu removal was evaluated in batch studies. For the preparation of fungal pellets, 7-days-old spore inoculum (10 %) of each strain was inoculated into sterile 50 ml Potato Dextrose Broth in a 250 ml conical flask and incubated for 96 hour at 30 ^{o}C in an orbital shaker (122 rpm). Mycelial pellets were filtered through cheesecloth. These fungal pellets (2.0 g wet weight) were added into 10% diluted effluent (pH 3.0) in 250 ml conical flask and incubated at 30 ^{o}C for 24 h at 122 rpm. The control was also run in parallel containing diluted effluent without fungal pellet. After 24 h of contact time, samples were centrifuged, filtered and supernatant was checked for residual Cu metal ions concentration. The potential strain with maximum Cu removal rate was selected for further work.

2.3 Identification

All isolates were identified on the basis of their morphological characteristics (colonial morphology, color, texture, shape, diameter and appearance of colony,) and microscopic characteristics (septation of mycelium, shape, diameter and texture of conidia).

2.4 Isolation of cell wall

The cell wall fraction of isolated fungal strains was separated by the method of Baik *et al*, 2002. Fungal mycelium (20.0 g) was homogenized for 15 min in a blender (National, Japan). Homogenized mycelium was washed with water and centrifuged for 30 min at 8000 rpm. The pellet was mixed with 250mL of mixture of chloroform and methanol (1:1). Then it was washed twice with acetone, and once with ethanol. The cell wall yield was 254.65 mg, 287.02 mg, 246.75 mg, 310.8 mg and 280 mg from 1.0 g fungal biomass of *Penicillium* sp., *Rhizopus sp, Mucor* sp., *Gliocladium viride* ZIC_{2063} and *Aspergillus* sp. The biosorption potential of cell wall component was determined. The cell wall (2.0 g) was suspended in 50 ml effluent in 250 ml conical flask and incubated at 30 ^{o}C for 24 h at 122 rpm.

2.5 FTIR spectrometer analysis

To characterize the Infrared spectra of fungal sp. Fourier Transform Infrared Spectrometer M 2000 series (MIDAC Corporation, Irvine California) was used.

2.6 Analytical Method for copper (VI)

The heavy metal concentration was determined by the use of Polarized Zeeman Atomic Absorption Spectrophotometer Z-5000 (Perkin Elmer Analyst 300 Hitachi, Japan). Determination of copper was done by using its specific lamp and at a specific wavelength. Samples (almost 5 ml each) of first 1h biosorption taken at predetermined interval were centrifuged and filtered. The supernatant were analyzed for Cu concentration.

The amount of metal bound by the biosorbents was calculated as follows.

$$Q = V (Ci- Cf) / m$$

Where,

Q = Metal uptake (mg metal per g of biosorbent),

V = Liquid sample volume (ml),

Ci = Initial concentration of the metal in the solution (mg/L),

Cf = Final concentration of the metal in the solution (mg/L) and

m = Amount of the added biosorbent on dry basis (mg).

3. Results and discussion

A total of fifty fungal strains were isolated from tanning effluent. Among these 11 isolates were of *Penicillium* sp., 7 of *Aspergillus* sp., 17 of *Gliocladium* sp., 6 of *Rhizopus* sp. and 9 isolates of *Mucor* sp. Pollution of water by heavy metals may lead to decrease in microbial diversity and enhanced the growth of resistant species (Ezzouhri *et al*., 2009). The data of table 1 also showed the metal tolerance index of the isolates. *Mucor* sp. were found to be very much sensitive to Cu which shows that Cu is highly toxic to its growth while thick mycelium of *Gliocladium viride* was observed in the presence of Cu than in control.

Among all fungal species *Gliocladium viride* showed higher efficiency to remove Cu. The removal efficiencies of fungal biosorbents for Cudecreased in the order: *Gliocladium* sp. > *Penicillium* sp. > *Aspergillus* sp. > *Rhizopus* sp. > *Mucor* sp. (Table.2). The statistical analysis of the data showed high significant level (89.48) for Cuuptake by *Gliocladium* viride ZIC_{2063} as compared to other isolates. The Curemoval efficiency of *Gliocladium viride* ZIC_{2063} (91.97 %) was much greater than other biosorbents reported by other researchers such as *Rhizopus nigricans* 80 % (Bai and Abraham, 2001), *Aspergillus niger* 83 % (Mala *et al*., 2006), *Rhizopus oryzae* 23 % (Park and Park, 2005) and *Sargassum* sp. 60 % (Cossich *et al*., 2002).

FTIR spectrum of all isolated fungi was also studied. FTIR spectrum of *Gliocladium virid* ZIC_{2063} has absorption peaks at 3922, 3467, 2393, 2354, 2055, 1634, 1072 and 517 cm^{-1} frequency level (data not shown). Absorption peaks of *Gliocladium viride* ZIC_{2063} indicates the presence of hydroxyl groups, Primary and secondary amines, amides stretching, Imines, oximes, C-OH stretching and C-N-C bonding are present on its cell wal (Fig 1)l. Biosorption process occurred at amino, hydroxyl and carboxyl groups present in cellulose and chitin of fungal cell wall (Ozsoy *et a*l., 2008). FTIR spectrum analysis of *Gliocladium viride* ZIC_{2063} showed that amine and its derivatives are the most common functional groups attached on its cell surface. Our results support the finding of other workers. According to Bai and Abraham, 2002 amino groups of fungal cell wall are mainly responsible for metal biosorption. metal ions did not bind to negatively charged functional groups such as carboxylate, phosphate and sulphate. Only positively charged amines (protonated at low pH) are responsible for binding of metal ions (Bayramoglu *et a*l., 2005).A single experiment was conducted to test the metal uptake capacity of whole mycelium and cell wall component (Table.2). Highest metal uptake (1230.2 mg) was found in cell wall component of *Gliocladium viride* ZIC2063. The cell wall components of other fungal isolates also gave greater metal uptake capacity than whole mycelium. It is in contrasted with other workers who reported greater metal uptake by whole mycelium than cell wall (Baik *et al*, 2002).

No.	**Isolates No.**	**Removal rate (%)**	**Cu tolerance index**
1	Penicillium TEI 1220	73.75	0.3
2	Penicillium TEI 1221	84.98	0.25
3	Penicillium TEI 1222	77.15	0.19
4	Penicillium TEI 1223	70.71	0.29
5	Penicillium TEI 1224	69.07	0.3
6	Penicillium TEI 1225	82.95	0.5
7	Penicillium TEI 1226	83.39	0.18
8	Penicillium TEI 1227	74.70	0.2
9	Penicillium TEI 1228	70.73	0.27
10	Penicillium TEI 1229	72.42	0.26
11	Penicillium TEI 1230	72.62	0.2
12	*Aspergillus* .ESGO2001	60.59	0.16
13	*Aspergillus* ESGO 2002	62.15	0.2
14	*Aspergillus* ESGO 2003	66.72	0.5
15	*Aspergillus* ESGO 2004	65.25	0.35
16	*Aspergillus* ESGO 2005	67.36	0.49
17	*Aspergillus* ESGO 2006	62.61	0.35
18	*Aspergillus* ESGO 2007	59.50	0.5
19	*Gliocladium* ZIC 2060	86.65	1.3
20	*Gliocladium* ZIC2061	85.71	1.39
21	*Gliocladium* ZIC 2062	89.75	1.41
22	*Gliocladium* ZIC 2063	96.98	1.42
23	*Gliocladium* ZIC 2064	79.74	1.38
24	*Gliocladium* ZIC 2065	91.60	1.32
25	*Gliocladium* ZIC 2066	87.49	1.4
26	*Gliocladium* ZIC 2067	83.03	1.38
27	*Gliocladium* ZIC 2068	79.61	1.31
28	*Gliocladium* ZIC 2069	87.69	1.33
29	*Gliocladium* ZIC 2070	83.29	1.36
30	*Gliocladium* ZIC 2071	84.94	1.39
31	*Gliocladium* ZIC 2072	92.43	1.4
32	*Gliocladium* ZIC 2073	90.45	1.32
33	*Gliocladium* ZIC 2074	82.84	1.35
34	*Gliocladium* ZIC 2075	91.48	1.37
35	*Gliocladium* ZIC 2076	86.60	1.35
36	*Rhizopus* SID 090	61.38	0.19
37	*Rhizopus* SSID 091	57.70	0.2
38	*Rhizopus* SSID 092	62.85	0.21

No.	Isolates No.	Removal rate (%)	Cu tolerance index
39	*Rhizopus* SSID 093	55.16	0.22
40	*Rhizopus* SSID 094	59.11	0.09
41	*Rhizopus* SSID 095	55.87	0.21
42	*Mucor* .CENTA 001	47.84	0.06
43	*Mucor* CENTA 002	51.01	0.04
44	*Mucor* CENTA 003	48.46	0.056
45	*Mucor* CENTA 004	50.36	0.06
46	*Mucor* CENTA 005	49.08	0.05
47	*Mucor* CENTA 006	45.50	0.09
48	*Mucor* CENTA 007	49.35	0.08
49	*Mucor* CENTA 008	50.62	0.1
50	*Mucor* CENTA 009	54.86	0.09

Biosorption Conditions: Incubation time 24 h, Temperature 30 ^{o}C, pH 3.0, Biosorbent 2.0 g (wet weight), agitation 122 rpm, Volume of reaction mixture 50 ml.

Table 1. Biosorption potiental of fungal isolates for their Cu removal efficiencies

Fig. 1. FTIR spectrum of *Gliocladium viride* ZIC$_{2063}$.

Sr.No	Organism	Biosorbent material	Cu binding capacity (mg/g)
1	*Penicillum sp.*	Whole mycelium	254.7
		Cell wall	964.5
2	*Aspergillus sp.*	Whole mycelium	126.7
		Cell wall	894.6
3	*Gliocladium sp.*	Whole mycelium	474.5
		Cell wall	1230.2
4	*Rhizopus sp.*	Whole mycelium	98.8
		Cell wall	726.2
5	*Mucor sp.*	Whole mycelium	94.6
		Cell wall	541.8

Table 2. Cubinding capacity of cell components of different fungal isolates.

4. Conclusion

In this study, copper resistant fungi were isolated from heavy metal contaminated environments, and the applicability of their heavy metal removal from industrial wastewater was evaluated at a laboratory scale. The heavy metal removal was determined for each isolate. *Gliocladium viride* was found to be highly copper tolerant fungus and exhibited thick growth than other fungal sp ecies. It appears that *Gliocladium* species has greater Cu removal efficiency (96.98 %) than other fungal species. Our findings also indicate direct relationship between level of metal resistance and biosorption capacity. Further investigations are underway to optimize the conditions for Cu removal from industrial effluent. *Gliocladium viride* ZIC_{2063} can be exploited as potential biosorbent for Cu from electroplating effluent.

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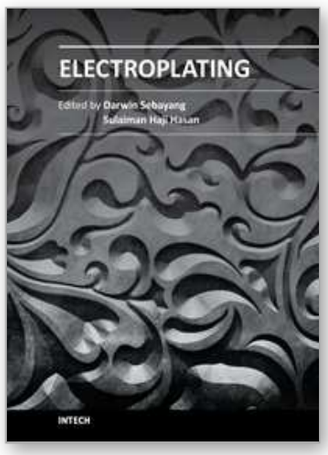

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This book emphasizes on new applications of electroplating with consideration for environmental aspect and experimental design. Written by experienced expert from various countries, the authors come from academia and electroplating industrial players. Here, a very detailed explanation to the new application of the electroplating is followed by a solution of the environmental issue caused by the electroplating process and concluded by experimental design for optimization of electro deposition processes.

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