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Research Article

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Metal tolerance and potential of *penicillium* species for use in mycoremediation.

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ABSTRACT

A cadmium resistant fungal strain belonging to the genera penicillium was obtained by carrying out successive enrichments from soil samples collected near a metal processing industry in Mumbai. The culture demonstrated resistance to 1.4 mg/ml cadmium. Optimum pH and temperature growth conditions for the isolate were determined. Study of growth pattern of this culture revealed a low specific growth rate, with an extended lag period in the presence of cadmium. Screening for resistance to other heavy metals showed significant tolerance to zinc, lead, nickel and copper. The isolate was found to remove 67%, 84%, and 95 % of cadmium from solution after 48, 72 and 96 hours respectively. This study highlights the important role that penicillium cultures exhibiting high metal tolerance, could play in bioremediation of polluted environments.

Key words: Tolerance, cadmium, *penicillium*, mycoremediation, heavy metals.

INTRODUCTION

Several investigators have reported contamination of soil and natural aquatic receptors due to heavy metals in effluents generated from various industries [1, 2]. Release of heavy metals into the environment, without proper treatment, poses a serious threat to public health, because of their persistence, biomagnification and bioaccumulation in the food chain. A number of studies have elaborated on the toxic effects of heavy metals on animals, plants and human health [3, 4].

Cadmium is extensively used in industry for a number of applications, including electroplating and stabilizing plastics and batteries. It is considered to be the third most dangerous heavy metal in water by the Environmental Protection Agency. Cadmium can get deposited in organs like kidneys, pancreas & liver, resulting in kidney necrosis and vomiting.

Conventional wastewater treatment technologies are very expensive and have several disadvantages, such as unpredictable metal ion removal, high reagent requirements and generation of toxic sludge, which are often difficult to dewater and require extreme caution during disposal [5]. Another major drawback is the lack of ability for removal of heavy metals in low concentrations, especially when the concentrations are in the 1–100 mg/L range.

It has been observed that microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and develop resistance [6]. This ability of the microorganisms to grow in the presence of heavy metals has a potential use in bioremediation of polluted waters [7]. Recently microbial systems like fungi, bacteria and algae have been explored for their role in the removal of heavy metals from polluted environments [8, 9]. Mycoremediation is a form of bioremediation using fungi to degrade or sequester contaminants in the environment. Fungi are present in aquatic sediments, terrestrial habitats and water surfaces and play a significant part in natural remediation of metal and aromatic compounds. Fungi also have advantages over bacteria since their hyphae can penetrate contaminated soil, to reach heavy metals. Despite the abundance of such fungi in contaminated niches,

penicillia in particular have received little attention in bioremediation and biodegradation studies. Additionally, studies conducted with different strains of imperfecti fungi, *Penicillium* spp. have demonstrated their ability to tolerate heavy metals and could be potentially interesting for the development of economically feasible processes for pollutant transformation.

In the present investigation, we isolated an indigenous fungal strain from a polluted site, through successive enrichments and evaluated its resistance to cadmium as well as other heavy metals. The cadmium removal ability of the fungal culture was assessed. This isolate was further characterized to evaluate the optimum pH and temperature conditions for its growth.

EXPERIMENTAL SECTION

Study area and sample collection

Soil samples were collected near a metal processing industry located near Dockyard Road in south Mumbai, Maharashtra, India (Figure1). From each site, five samples of the upper soil layer (not exceeding a depth of 5 cm) were taken and mixed to form a composite sample which was transferred into a sterile container and immediately transported to the laboratory for further studies.

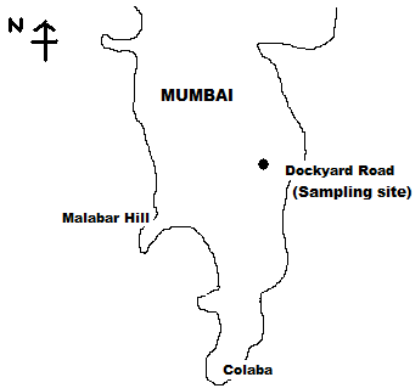

Fig 1: Location of the sampling site in Mumbai.

Culture media

Fungal strains were isolated from the soil samples using yeast extract-peptone-dextrose (YEPD) medium. The medium was prepared by dissolving 1 g of yeast extract, 0.5 g peptone, and 0.2 g glucose in 100-mL distilled water; pH was adjusted to 7.2-7.5 and 1.5 g agar added. The medium was autoclaved at 121°C, 15 lb pressure for 15 min.

Isolation of cadmium resistant fungal culture

The conventional plate method was used to isolate cadmium-resistant fungal strains. Soil suspensions were inoculated into YEPD broth amended with 0.05 mg L^{-1} cadmium. Cultures were incubated on a rotary shaker at 30°C, 180 rpm for 3 - 7 days and then spread on agar plates supplemented with cadmium. In order to isolate strains with high cadmium tolerance, successive enrichments were carried out by gradually increasing the concentration of cadmium in the medium from 100 mg L^{-1} to 2000 mg L^{-1}. The fungal culture showing highest cadmium resistance was selected for further study.

Identification of the Fungal Isolate

The isolated fungal culture was identified based on morphological characteristics and microscopic observation.

Determination of Optimum Growth conditions

For determination of optimum growth temperature, sets of tubes containing 5 mL YEPD broth were inoculated with 0.1ml of the fungal isolate. These tubes were incubated at 4°C, 25°C, 37°C, and 55°C. After incubation for 48 h, absorbance was measured at 530 nm. (Erma Inc. Colorimeter).

Optimum pH for the growth of the fungal isolate was determined by inoculating tubes of YEPD broth with the pH ranging from 4 to 10. After an incubation period of 48 h, absorbance was measured at 530 nm.

Growth Curve of the fungal isolate

Effect of cadmium on the growth of the fungal isolate was determined by inoculating it in YEPD medium supplemented with 350 mg L^{-1} of cadmium (sub lethal concentration). These flasks were incubated at 30°C on a shaker at 60–80 rpm. Aliquots of the culture were taken at regular intervals over a period of 96 hours and absorbance measured at 530 nm. Controls were maintained and consisted of inoculated medium without the supplementation of cadmium ions.

Cross Metal Resistance assays

Cross heavy metal resistance of the fungal isolate was determined using stock solutions of 4 mg mL^{-1} of different metal salts (cadmium chloride, copper sulfate, lead nitrate, nickel chloride, mercuric chloride, potassium dichromate, and zinc sulfate). Various dilutions of the stock solution were prepared using YEPD broth and 0.1 ml of 48 hr old culture added. Tubes were incubated for 48 hrs and the lowest concentration of the heavy metal that did not show growth corresponded to the minimum inhibitory concentration. Positive and negative controls were maintained.

Estimation of Cadmium Processing Ability of the Yeast Isolate

The metal processing capability of the yeast isolate was checked by adding Cd^{2+} at a concentration of 100 ppm in the defined culture medium (gram per liter): D-glucose 30; yeast autolysate 7.5; peptone 7.5; NH_4Cl 9; KH_2PO_4 2.75; $MgCl_2 \cdot 2H_2O$ 2; $FeSO_4 \cdot 7H_2O$, 0.002; K_2HPO_4 5.2; (pH 7.2–7.5); to minimize the complexation of the heavy metal ions [10]. A control culture medium containing the same concentration of cadmium as in the treated one, i.e. 100 ppm but without the yeast isolate was also maintained. Cultures were incubated at 30°C for 96 h, and from each medium (control and treated), 5 mL culture was taken out under sterile conditions after 48, 96, and 144 h, respectively. Cultures were centrifuged at 3,000 rpm for 5 min. The pellets [acid digested, 0.2 N HNO3 (1:1)] and supernatants were used for the estimation of cadmium by atomic absorption spectrophotometer (AAS 7000, Shimadzu.). In the present study, metal uptake values were determined from the difference in the final metal concentration between control flask without cells and test flask with cells at different time periods. The amount of metal in the pellets and supernatants was determined by using a standard curve. The percentage decrease in the amount of cadmium in the medium was calculated.

Experiments were performed in triplicate and repeated three times. Mean values have been reported.

RESULTS AND DISCUSSION

Release of heavy metals into the environment, has increased globally due to rapid industrialization, posing a significant threat to the environment and public health.

Heavy metals contaminating soil and water include cadmium, lead, chromium, copper, mercury and nickel, all of which are extremely toxic to biological systems and bioaccumulate in them [11,12]. Today, numerous consumer products have also been reported as a source of heavy metal exposure to human beings. [13] Among heavy metals that have a long biological half-live, cadmium, in particular, constitutes a major problem in industrialized nations [14]. Cadmium is highly toxic to organisms even at very low concentrations. It inhibits DNA replication [15] and appears to make it more susceptible to nucleolytic attack [16]. Cadmium exhibits a strong affinity to glutathione and sulfhydryl groups in proteins and can cause cellular damage.

Generally, pollution of soil or water by heavy metals results in a decrease in the resident microbial population. The imposition of environmental stress, leads to the extinction of sensitive species and acts as selective pressure for the development of metal-resistant variants. These autochthonous microbes, which are extremely versatile and have survived in polluted environments can be isolated and explored for bioremediation of heavy metals. Recent studies have shown that strains isolated from contaminated niches have an excellent ability to remove significant quantities of metals from aqueous solutions [17]. The removal of toxic heavy metals to an environmentally safe level in a cost effective and environment friendly manner using indigenous microorganisms has assumed great importance. Fungi are known to be more tolerant to metals and have a higher surface to volume ratio than bacteria or actinomycetes. Fungi are not only a major component of the biota in soils and mineral substrates, but also under certain environmental conditions (low pH), can be efficient biogeochemical agents and bioaccumulators of soluble and particulate forms of metals. Some fungi which have previously been isolated and explored for bioremediation of cadmium include *Phanerochaete chryssporium* [18, 19], *Penicillium oxalicum var. Armeniaca*, *Tolypocladium* species [20] and species of *Aspergillus* and *Rhizopus* [21]. Among these fungal isolates, belonging to *penicillium* spp. have been described as prominent ones [22].

The current study was carried out to obtain a heavy metal resistant fungal strain from contaminated soil and test its potential for removal of cadmium from solution. We obtained a fungal culture which showed resistance to 1.4

mg/mL of cadmium by carrying out successive enrichments. Fungal colonies growing on YEPD agar amended with cadmium after 4 days had green filamentous mycelia and displayed delayed conidiogenesis. They were identified as belonging to the genera *Penicillium* based on morphological characteristics and microscopic observation.

With increasing concentration of cadmium in the medium, the time required for the fungus to grow increased significantly. This was evident from the pattern of the growth curve obtained by growing the culture in the presence of sub-lethal concentration of cadmium (350µg/ml) and comparing it with the control culture in which no metal ions were added. The lag phase of the *penicillium* isolate was significantly extended in cadmium treated medium. A reduction in the growth rate is a typical response of fungi to toxicants [23], though a lengthening of the lag phase may not always occur. Jones and Hutchinson (1988) [24] reported an increase in the lag time among different *Basidiomycete* species cultivated on zinc and cadmium amended media, while other investigators have reported the absence of any correlation of cadmium concentration with the lag time for other fungal cultures [25] .

In the pH range studied (4 to 10), optimum growth was observed around pH 6. The optimum temperature for the growth of the isolate was found to be about 37^0C, which was slightly higher compared to the temperature used when enriching and isolating the culture, suggesting that the selection of these isolates was influenced by the presence of the heavy metal in the medium and not by the temperature used in the isolation procedure.

Since microbiota isolated from co-contaminated environments have been reported to exhibit resistance to more than one metal ions [26], tolerance experiments to other heavy metals like lead, copper, mercury, nickel and chromium were also conducted. The wide resistance to heavy metals is of importance in view of fact that it could contribute to better application of the fungal culture for bioremediation of polluted environments.

The isolate possessed marked resistance to zinc (3.2 mg/ml), lead (2mg/ml), mercury (1.2mg/ml) and copper (1.2mg/ml). Low tolerance of the isolate was observed towards nickel (0.8 mg/ml) and chromium (less than 0.4 mg/ml). The order of resistance to heavy metal concentration was Zn > Pb > Cd > Cu = Hg > Ni > Cr. Variation in heavy metal tolerance in *Penicillium* species has also been reported by other investigators [27] and may be due to the presence of different types of resistance mechanisms exhibited by this culture.

In order to allow for a better understanding of the potential of the *Penicillium* isolate, for in-field bioremediation processes, the culture was tested in a setting miming real environmental exposure by inoculating it in a medium supplemented with 100 ppm of cadmium. The extent of removal of the heavy metal from solution was determined at various time intervals by atomic absorption spectroscopy. Total cadmium uptake (adsorption and absorption) was 67, 84, and 95 mg L−1 from the medium after 48, 72 and 96 hours respectively .In our study the *penicillium* isolate could remove 95% of cadmium from the medium after 96 h of incubation In the same context, Hemambika B.et al (2011) [28] reported the potential of *penicillium* species isolated from Tamil Nadu, India to remove 97.21% of cadmium after immobilization.

In conclusion, our study suggests that the isolated *penicillium* culture showed a high level of resistance to most of the heavy metals tested, which could make it an attractive potential candidate for further investigations, for removal of heavy metals from contaminated environments.

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REFERENCES

[1] L. Friberg, Proceedings of the International Conference on Heavy Metals in the Environment, **1975,** vol. 1, pp. 21–34.
[2] F.A., Nicholson S.R. Smith, B.J. Alloway, C.Carlton-Smith, B.J. Chambers. *Science of the Total Environment.* **2003**. Vol 311, Issue 1-3, pp 205-219.
[3] Y.Chisti, *Biotechnology Advances*, **2004**. 6, 431–432.
[4] M.Coeurdassier, A.Devaufleury, , R.Scheifler, , E. Morhain, & P.Badot; M. *Bulletin of Environmental Contamination and Toxicology*, **2004.** 72, 1083–1090.
[5] S. I. Amer. *Environmental Technology*; **1998**. pp.1-7.
[6] B.Prasenjit, S. Sumathi, *J. Mater. Cycles Waste Manag.* **2005**. 788–92.
[7] A. R. Shakoori, A.Rehman, & R. U Haq. *Bulletin of Environmental Contamination and Toxicology*, **2004**. 72, 1046–1051.
[8] E. Terres, A. Cid, C. Herrero, J. Abalde. *Biores. Technol*. **1998**. 63 213–220.

[9] Natarajan S T, Jayaraj R. Thanaraj P J et al. *J. Chem. Pharm. Res.*, **2011,** 3(2):595-604.
[10] P.Kujan, A.Prell, H.Šafar, M.Sobotka, T. Řezankat, & P. Holler, *Folia Microbiologica*, **2005.** 50, 309–313.
[11] A. V. Mane, R. R. Sankpal, L. A. Mane, M. S. Ambawade .*J. Chem. Pharm. Res.*, **2010**, 2(5): 206-215
[12] Mukke V K., *J. Chem. Pharm. Res.* **2012**, 4(1):398-401.
[13] A. S. Chauhan, R. Bhadauria1, A. K. Singh, S.S. Lodhi, K. D. Chaturvedi,V. S. Tomar. *J. Chem. Pharm. Res.*, **2010**, 2(6):92-97.
[14] RA Tilaki, AH Hahvi, M Shariat, S Nasseri. *Iranian J. Public Health*. **2004**.33(4): 43-52.
[15] T Nystr¨Om, S.Kjelleberg, *FEMS Microbiol.Ecol.* **1987**.45: 143–153.
[16] R. S. Mitra, I. A. Bernstein, *Biochem. Biophys. Res. Commun.* **1977.** 74: 1450–1455.
[17] A Malik. *Environ. Int.* **2004** 30: 261-278
[18] R Say, A Denizli, AM Yakup. *Bioresour. Technol.* **2001**.76: 67-70.
[19] Q. Li, S. Wu, G. Liu, X. Liao, X. *Sep. Purif. Technol*. **2004.** 34: 135-142.
[20] L Svecova, M Spanelova, M Kubal, E Guibal. *Separation Purification Technol*. **2006.**52(1): 142-153
[21] S Zafar, F Aqil, I Ahmad. *Bioresour. Technol*. **2007**.98: 2557-2561.
[22] H Niu, XS Xu, JH Wang, B Volesky. *Biotechnol. Bioeng.*; **1993**. 2:785–787.
[23] GM Gadd. *New Phytol*. **1993**.124: 25-60.
[24] MD Jones, TC Hutchinson *Can. J. Bot*. **1988**.66: 119-124
[25] A.B.Darlington ,W.E. Rauser *Can.J.Bot.* **1988**66:225-229.
[26] GM Gadd, GM Sayer .*American Soc. Microbiol*. **2000**. pp. 237-256.
[27] L.Ezzouhri, E.Castro, M.Moya, F Espinola , K Lairini. *African Journal of Microbiology Research* **2009**.Vol. 3 (2) pp. 035-048.
[28] B.Hemambika, M.Johncy Rani , V Rajesh Kannan . *Journal of Ecology and the Natural Environment.* **2011**. Vol. 3(5), pp. 168-175.