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Isolation and characterisation of different Chromium (VI) resistant Fungal sp from industrially polluted sites

R. Vaideeshwaran, J. Jayachitra and P. Sivasakthivelan

Department of Agricultural Microbiology, Faculty of Agriculture
Annamalai University, Annamalai Nagar – 608002 (India)
Corresponding Author: microjayachitra@gmail.com

Abstract

Heavy metal contamination in soil is a rising problem across the globe due to industrial, mining, agricultural, and other activities. Fungi are the most prevalent and efficient heavy metal resistant microbial family with metal bioleaching ability. The present study aimed on the isolation and characterization of the heavy metal resistant fungal strains from polluted soil. All the strains were grown on PDA medium and Macro and Microscopic characteristics of all the strains were observed. Five strains were isolated and they were identified as *Aspergillus niger* (AN-1 & AN-2) *Aspergillus fumigatus* (AF-3 & AF-4) and *Trichoderma virens* (TF-5). Screening of chromium resistant fungal isolates was examined by poison food technique. Among the five fungal strains isolated *Aspergillus niger* (AN-2) recorded highest mycelial growth rate (66.0mm) at 50ppm of Chromium ions. *Aspergillus fumigatus* (AF-4) recorded lowest mycelial growth rate (38.8mm) at 200ppm of Chromium ions.

Key words : Chromium resistant fungi, *Aspergillus niger*, *Aspergillus fumigatus* and *Trichoderma virens*.

Industrialization is one of the reasons for the toxic heavy metals are addition to the environment. Heavy metal mobility, toxicity, and bioavailability must all be lowered to solve this issue[10]. Chromium is the seventh most prevalent element on the world and appears in a variety of oxidation states. The two most common types of chromium in the environment are hexavalent and trivalent[2]. Chromium is one of the most poisonous heavy metals, posing health risks to plants and animals. Many industrial processes emit Chromium into the atmosphere, including chrome plating, petroleum refining, leather tanning, wood preservation, textile manufacture, and pulp processing[4]. If the quantity of Cr(VI) in the environment surpasses >0.05 mg/L, it may disrupt human physiology and, if it enters the food chain, it create significant health concerns such as irritation in skin, nasal irritation, ulceration, eardrum perforation, and lung cancer[11,12].

Further, it affects the eyes, kidneys,

respiratory system, skin, and liver[5]. The key risk factor for chromium poisoning is environmental exposure; due to the nature of their work environment, industrial welders are at a high risk of this exposure. When a compound containing hexavalent chromium comes into contact with the skin, it produces rashes and ulcers. There are multiple techniques Viz., filtration, chemical precipitation, reverse osmosis, membrane technology, oxidation and reduction, ion exchange, and electrochemical treatment, for the removal of heavy metals from a contaminated environment. However, there are some significant disadvantages to these techniques. The most crucial one is that heavy metals that are present in lower concentrations (less than 100 mg/L) cannot be eliminated[3]. Although the features of the soil may alter as a result of these techniques, the heavy metal pollution can still be there. They are also expensive and do not require any energy. Additionally, the toxin can spread to other areas of the ecosystem where it could accumulate and cause the same issue[8]. Nowadays, biosorption is identified as promising alternative for the removal of heavy metals at low concentrations (<100 ppm), including chromium. This method requires low operation costs, is easy to implement, availability, low cost, and eco-friendly nature. The aim of this present study to isolate and characterize Chromium resistant the fungal strains from chromium polluted soil.

Sample collection :

Soil samples were collected in sterile collection bottles from various locations of chromium polluted soil in Vellore District, Tamil Nadu. The samples were stored at ice box and immediately transferred to the laboratory for further analysis.

Isolation and characterization of Aspergillus sp and Trichoderma sp.

Aspergillus and *Trichoderma* fungi were isolated from chromium polluted soil sample by using PDA medium by a dilution plate method. The soil sample was serially diluted up to 10^{-4} dilution and pipette out 1 ml of the sample from 10^{-4} dilution and transfer into sterile petriplates. Pour about 10 to 15 ml of the PDA medium into each petriplate, rotate well for the even distribution of the medium and incubate at 25-28ºC for 7 days. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). An individual colony was isolated from the same plates and subculture onto a fresh Potato Dextrose Agar (PDA) plate. Distinct morphological characteristics were observed for identification and the plates were stored at 4°C. Visual observation on petridishes and micro- morphological studies in slide culture, were adopted for the identification of *Aspergillus and Trichoderma* sp.

For visual observation, the isolates were grown on PDA agar for 3-5 days. The mode of mycelia growth, colour and changes in medium colour for each isolate were examined daily. For micro and morphological studies, a slide culture technique was used[6]. Examination of the shape, size, arrangement and development of conidiophores or phialides provided a tentative identification of *Aspergillus and Trichoderma* sp. Samples were compared to a taxonomic key for the genus *Aspergillus and Trichoderma* sp. The culture is purified by single spore isolation or single hyphal tip

method and transferred into PDA slants and kept at 4ºC for further use. The isolates were named as AN-1, AN-2, AF-3, AF-4 and TF-5.

Screening of chromium tolerant fungal strains :

In vitro tolerance of fungal strains such as Viz., *Aspergillus niger (AN-1 & AN-2) Aspergillus fumigatus* (AF-3 & AF-4) and *Trichoderma virens* (TF-5) to different concentration of chromium was determined by the poisoned food technique. PDA medium (100ml) was prepared in 250 ml conical flask, then appropriate quantities of chromium stock solutions were added to molten PDA to get the required concentrations (0,50,100,150 and 200 mg/l) and the resulting media were poured into Petri plates after gentle shaking. The non-amended medium of chromium served as the control. The plates were inoculated by placing 6 mm mycelial discs of 4day old cultures of the fungal strains such as *Aspergillus niger* (AN-1 & AN-2) *Aspergillus fumigatus* (AF-3 & AF-4) and *Trichoderma virens* (TF-5) on the agar surface and incubated at 28 ± 1ºC for 3-7 days. Isolates showing maximum radial growth on the media, irrespective of the metal concentration, were selected for further studies.

Isolation and characterization of Aspergillus sp and Trichoderma sp.:

In the present study five fungal strains were isolated and they are identified based on the Macro and Microscopic methods. The isolated fungal strains AN-1 and AN-2 were showed the colony colour initially as white to yellow and then turned to black. Later the colony gradually became black molds with cottony appearance visual to the naked eye. The conidiophores are protrusions from a septate and hyaline hyphae. The conidial heads appear radial and they split into columns (biseriate). The conidiophore vesicle produces sterile cells known as metulae which support the phialides on the conidiophores, The Conidiophores are 400-3000 µm long, they are smooth and hyaline. The conidiophore becomes dark at the apex and terminating in a globose vesicle which is 30-75 µm in diameter.

The metulae and phialides cover the vesicle. The phialides produce conidia that have a rough texture, with dark brown color, and have a diameter of 4-5µm and it is identified as *Aspergillus niger*.

The isolates AF-3 and AF-4 showed initial colony colour as green spiked conidia i.e the surface has small spikes covering its surface. The conidia are 2.5-3 µm in diameter. The conidia have a smooth surface or spiked (spinose). Conidia were produced in column chains that are basipetal (facing downwards) from green phialides of 6-8 by 2-3 µm in size. Strains produce white coloured conidia because they lack pigment. Conidial chains are produced directly on broadly clavate vesicles (20-30 µm in diameter) in the absence of metulae (one of the outermost branches of a conidiophore from which flask-shaped phialides radiate) and it is identified as *Aspergillus fumigatus*.

The isolate TF-5, initially showed white to light green colony colour, watery in centre. Later the colony gradually turned deep grass green in colour and looked soft and leathery to the naked eye. The conidiophores were erect, smooth, penicillately branched,

asymmetrical branches singly or vertically arranged at different levels, phialides were flask-shaped, coverage toward the main branch, emphasizing the penicillate branching. Phialospores were sub-globose to elliptical, smooth-walled and it is identified as *Trichoderma virens*. These result are in accordance with above findings of Ahsanur Rahman,[1] he has also reported the similar characteristics of *Trichoderma* isolated from different habitats. Oluwafemi *et al.,*[7] isolated different strains of fungal species which were identified as *Aspergillus niger, Penicillium notatum and Aspergillus favus* from copper, chromium, cadmium and nickel polluted soil in China.

Table-1. Macro and Microscopic Characterization of fungal strains from Chromium polluted soil.

Isolate Number	Macroscopic characters	Microscopic characters	Identified as
(Aspergillus niger) AN-1 and AN-2	• Wooly • At first white to yellow than turning black • Reverse white to yellow	Conidiophores • Long smooth (400-3000µm) Phialides • Biseriate • Cover entire vesicle • Form "radiate" head	*Aspergillus niger*
(Aspergillus fumigatus) AF-3 and AF-4	• Velvety or powdery • At first white than turned to dark greenish to gray with a narrow white border. • Reverse white to tan	Conidiophores • Short smooth (<300µm) Phialides • Uniseriate • Usually only on upper two- third of vesicle • Parallel to axis of Conidiophores	*Aspergillus fumigatus*
	• Culture are fast growing at 25-30° C • Conidia forming within on week in compact or loose tufts in shades of green or yellow or less freq uently white	Conidiophores • Highly branched, difficult to define or measure. • Conidia loosely or compactly tufted	

(*Trichoderma virens*) TF-5	• Yellow pigment may be secreted in to the agar, specially on PDA • A characteristic sweet or 'coconut' odour is produced by some species.	• Main branches of the Conidiophores produce lateral side branches. • The branches may rebranch, with the secondary branches being closest to main axis. Conidia • Typically appear dry but in some species they may be held in drops of clear green or yellow • Round in oval in shape.	*Trichoderma virens*

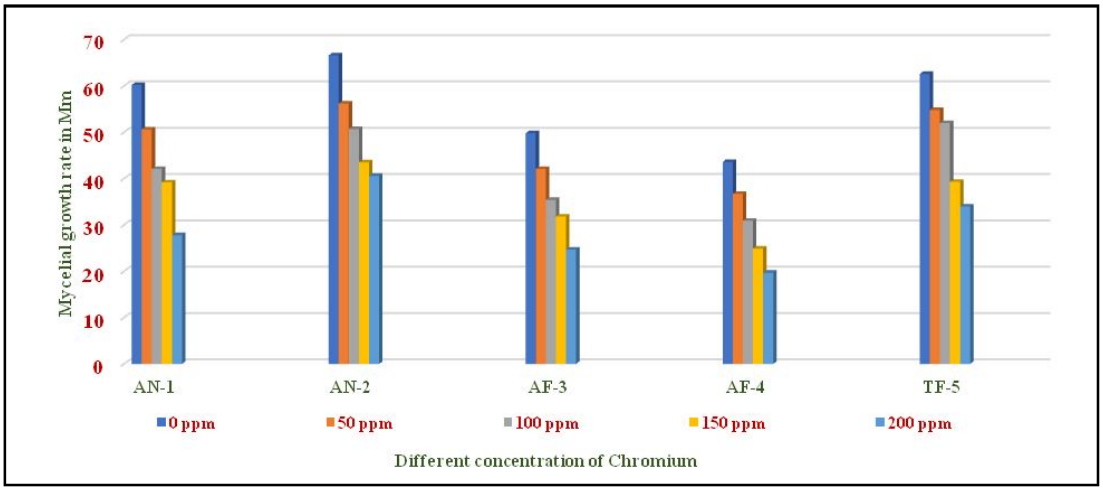

Fig. 1. Effect of Chromium concentration on various fungal sp isolated from soils

Screening of chromium tolerant fungal strains isolated from chromium polluted soil :

Screening of the *Aspergillus and Trichoderma* sp isolates for their chromium tolerance revealed that the isolates varied significantly in their levels of tolerance, regardless of the tested chromium concentrations (Table-2). At 50 ppm of chromium concentration, the highest mycelial growth rate was recorded by AF-2 (56.2.0mm), which was significantly higher than that of TF-5 (54.8mm), followed by AF-1 (50.6mm). The data indicated that AF-2, TF-5 and AF-1 were tolerant, AF-3

Table-2. Screening of chromium tolerant *Aspergillus and Trichoderma* sp

Isolate Number	Growth rate (mm)				
	0 ppm (control)	50 ppm	100 ppm	150 ppm	200 ppm
AN-1 *(Aspergillus niger)*	60.2	50.6	42.1	39.2	27.8
AN-2 *(Aspergillus niger)*	66.6	56.2	50.7	43.5	40.6
AF-3 *(Aspergillus fumigatus)*	49.8	42.1	35.4	31.8	24.7
AF-4 *(Aspergillus fumigatus)*	43.6	36.7	30.9	24.9	19.7
TF-5 *(Trichoderma virens)*	62.6	54.8	52.0	39.3	34.0

were moderately tolerant, and AF-4 were susceptible to chromium toxicity (Table-2). In (Fig. 1) showed the highest mycelial growth rate was observed by *Aspergillus niger* (AF-2) 50 ppm (56.2mm) while increasing the concentration of chromium the mycelial growth rate decreased. Variations in metal tolerance among different species of a genus or within the same species might be due to the presence of one or more resistance mechanisms exhibited by different fungi. Sarkar *et al.,*[9] reported *Trichoderma harzianum* to be moderately tolerant to up to 60 ppm of Ni, at that concentration the level of inhibition of mycelial growth was 33.3%. A further increase in the Ni concentration reduced the growth, and total inhibition was observed at 200mg/L this results are inlined with the presence study. Oluwafemi *et al.,*[7] reported that the biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil.

In the present study, the Chromium tolerant fungal strains were isolated from chromium polluted soil. There were five fungal strains were isolated and it was identified on the basis of Macro/Microscopic observations. Among the five fungal strains two were identified as (*Aspergillus niger),* two (*Aspergillus fumigatus*) and one (*Trichoderma virens*) was identified. In screening of chromium tolerant fungal Isolate-2 *Aspergillus niger* was found to be recorded highest mycelial growth compared with other isolates.

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