In Search of Industrial Clean-up Clients; Evaluation of Heavy Metal Tolerability of Rhizospheric Trichoderma

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Bhavya G.
University of Mysore

Channarayapatna-Ramesh Sunil Kumar
MMK & SDM Mahila Mahavidyala, Mysuru

Swati K.
University of Mysore

Dr. Geetha N
University of Mysore

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Bhavya G[1]., Sunil K. C. R[1]., Nandini B[1]., Swati K[1]., Prakash H. S[1]. and Geetha N[1]*
[1]Eco-Biotech Laboratory
Department of Studies in Biotechnology
University of Mysore, Manasagangotri
Mysuru, Karnataka, India

Abstract

Evaluating tolerability of different *Trichoderma* species against various heavy metal salts was the prime intention of the current investigation. *Trichoderma virens*, *T. asperellum*, *T. atroviride*, *T. longibrachiatum* and *T. harzianum* isolated from paddy fields irrigated with industrial effluents were inoculated in potato dextrose broth amended with metal salts like potassium dichromate ($K_2Cr_2O_7$) as hexavalent chromium source, Mercuric chloride ($HgCl_2$) as source of mercury and cadmium chloride ($CdCl_2$) as cadmium source at different concentrations i.e. 0.4, 0.6, 0.8 and 1mM with optimal pH conditions. All the five isolates tested against metal have proven their potentiality towards accepting metal salts in their growth media. Results obtained from the studies has shown variance among species and their metal tolerance too.

Keywords: *Trichoderma,* Mercuric chloride, Cadmium chloride, Potassium di-chromate, Industrial effluents.

1. Introduction

Increased industrial activities during the recent years, agricultural practices and use of chemicals in day to day life under controlled and uncontrolled manner, release heavy metals and toxic chemicals into the environment [1, 2]. After metals pass into the ecosystem, they start damaging as they move from an ecological layer to another. Though the regulations were tough against disposal of metals, conventional technologies available at present are not effective enough or economic and also poor consideration of waste water quantities made scientists to search for better biological clean-up tools which is economical to adopt [3]. R&D literatures available till date mark the potential ability of microbes towards substantial removal of heavy metals and other xenobiotics from the aqueous solutions [4-6]. The interaction with the non-living components of the environment make *Fungi* as a key group of microorganisms in the regulation of ecosystem processes [7]. Exploration of fungi in clean-up technologies to remediate polluted ecosystem has not been utilized, and it tends to be neglected. As such, the potential use of fungi in bioremediation and waste treatment has not received the attention, though they are merited by the extensive metabolic capabilities [2]. Till date, as per the available literature the knowledge acquired towards understanding abilities of fungal strains to employ in the processes of bioremediation is poor. In the present study, paddy rhizospheric samples were screened to study diversity of *Trichoderma* sp. and their tolerance ability against different heavy metal salts in order to make an strategized effort in future investigations towards designing economically stable clean-up tool.

2. Materials and Methods.

2.1. Isolation and identification of *Trichoderma* species from the rhizospheric soil samples.

Rhizospheric soil samples collected from the paddy field of suburban Mysore, Karnataka state, India [8], was brought to the laboratory in sterilized polythene bags. Serially diluted samples were inoculated on Potato dextrose agar plates and incubated at room temperature for 3-4 days. The cultures were identified morphologically [9].

2.2. Determination of optimum pH of *Trichoderma* species.

Five isolates of *Trichoderma*, *T. virens*, *T. asperellum*, *T. atroviride*, *T. longibrachiatum* and *T. harzianum* were inoculated on to sterilized potato dextrose broth [PDB] with pH of 3, 5, 7 and 9 adjusted with 1N base and acid. After seven days of incubation, mycelial mat from the broth was filtered out using sterilized filter papers and dried at 85^0C. Dry mass of mycelial mat was estimated (Shimadzu, Japan). Experiments were conducted in triplicates.

2.3. Determination of Minimal Inhibitory Concentration (MIC) against differential heavy metal salts.

All the five *Trichoderma* sp. were inoculated in sterilized 100ml PDB amended with 0.0, 0.4, 0.6, 0.8 and 1.0 mM of $HgCl_2$, $CdCl_2$, and $K_2Cr_2O_7$ salts. Broth without metal salts was considered as control. After seven days of incubation period, mycelial mat from the broth was filtered out using sterilized filter papers and dried at 85^0C. Dry mycelial mass was estimated (Shimadzu, Japan). Experiments were conducted in triplicates.

3. Results and Discussion

3.1. Isolation and identification of *Trichoderma* species from the rhizospheric soil samples.

Environmentally acceptable new technologies are required to reduce heavy metal concentration at affordable costs. Hence, much attention has now been given for biosorption technology using microorganisms. The present study was aimed to investigate the heavy metal biosorption efficiency of *Trichoderma* species isolated from rhizospheric soil samples.

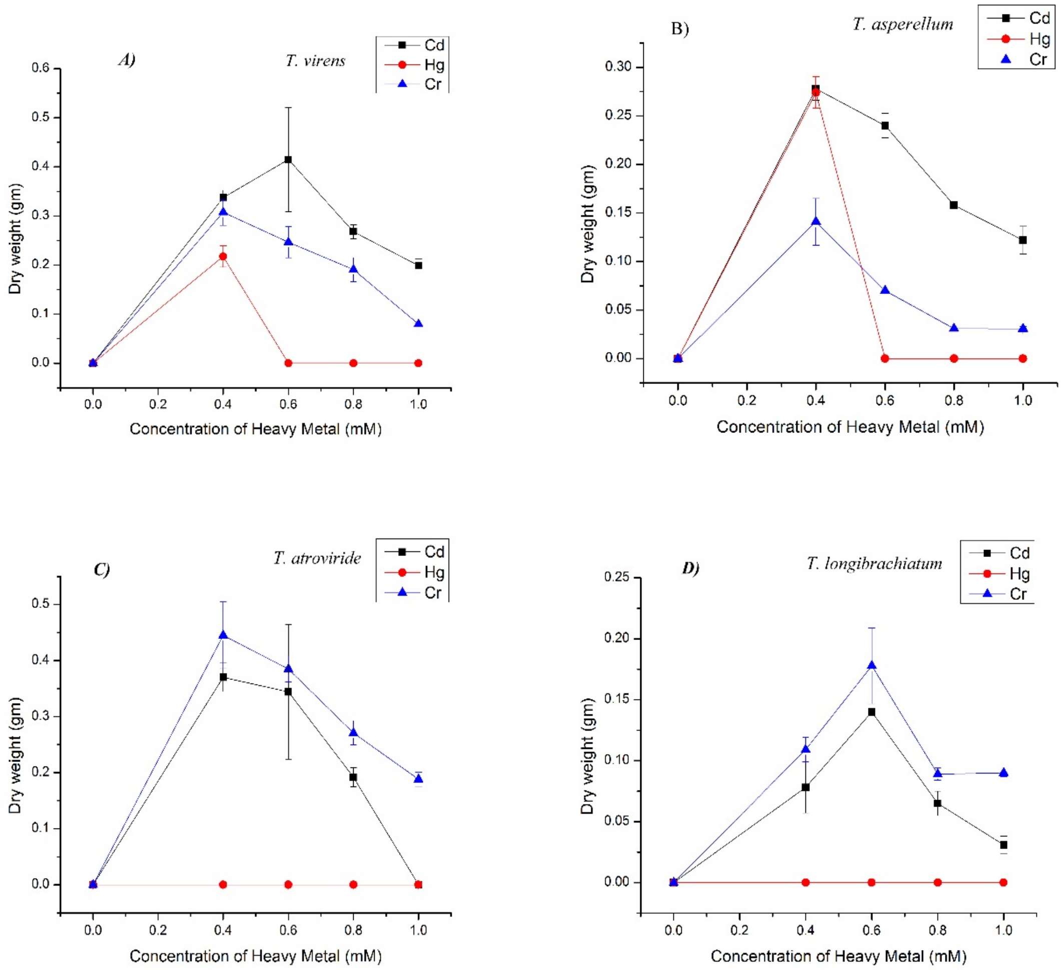
This investigation reveals the presence of diversified species of *Trichoderma* in paddy rhizospheric soil samples. The isolated *Trichoderma* species were found to be influenced by several factors like pH, initial heavy metal ion concentration, biomass dose, etc., the cultures grew well as their optimal pH at acidophilic conditions, in the growth medium.

3.2. Effect of pH

The effect of pH on biomass was studied at varying pH 3.0 to 9.0 at 25^0C. The fungal strains of *Trichoderma* sp., *T. virens, T. asperellum, T. atroviride, T. longibrachiatum* and *T. harzianum* grew well at their optimal pH at acidophilic range (Fig. 1. G). At low pH value, the H^+ ions compete with metal cation for the exchange sites in the system thereby partially releasing the metal cation [10]. The cell surface metal binding sites and metal chemistry in the solution is affected by pH. The anions dominantly present would be expected to interact more strongly with positively charged amines of the chitosan in *Trichoderma* cell wall.

3.3. Effect of initial heavy metal ion concentration

The biosorption of heavy metal on *Trichoderma* isolates (fungal biomass) was carried out at different initial heavy metal concentration 0.0 to 1.0mM (Fig 1. F). It was observed that though the *Trichoderma* strains were isolated from the same rhizospheric soil samples, they exhibited metal specificity along with differentiated growth rate against various concentrations of metal salts (Fig 1. A-E). There are variations in percent adsorption of heavy metals at different concentrations among the isolates. Among the five *Trichoderma* sp., *T. harzianum* has proven its tolerance capacity against all amended metal salts by growing at its optimum rate. *T. harzianum* has stable growth rate at 0.4, 0.6, and up to 0.8mM concentration and declined at 1.0mM concentration of Hg, whereas, *T. atroviride* failed to accept/adsorb Hg even at very low concentration and exhibited highest tolerance against Cr (VI) and Cd supplements in the medium. *T. virens* showed its maximal tolerance against Cd and least against Cr (VI) and Hg respectively. The isolates *T. asperellum* and *T. longibrachiatum* even failed to colonize at the lowest concentration of any of the salts.



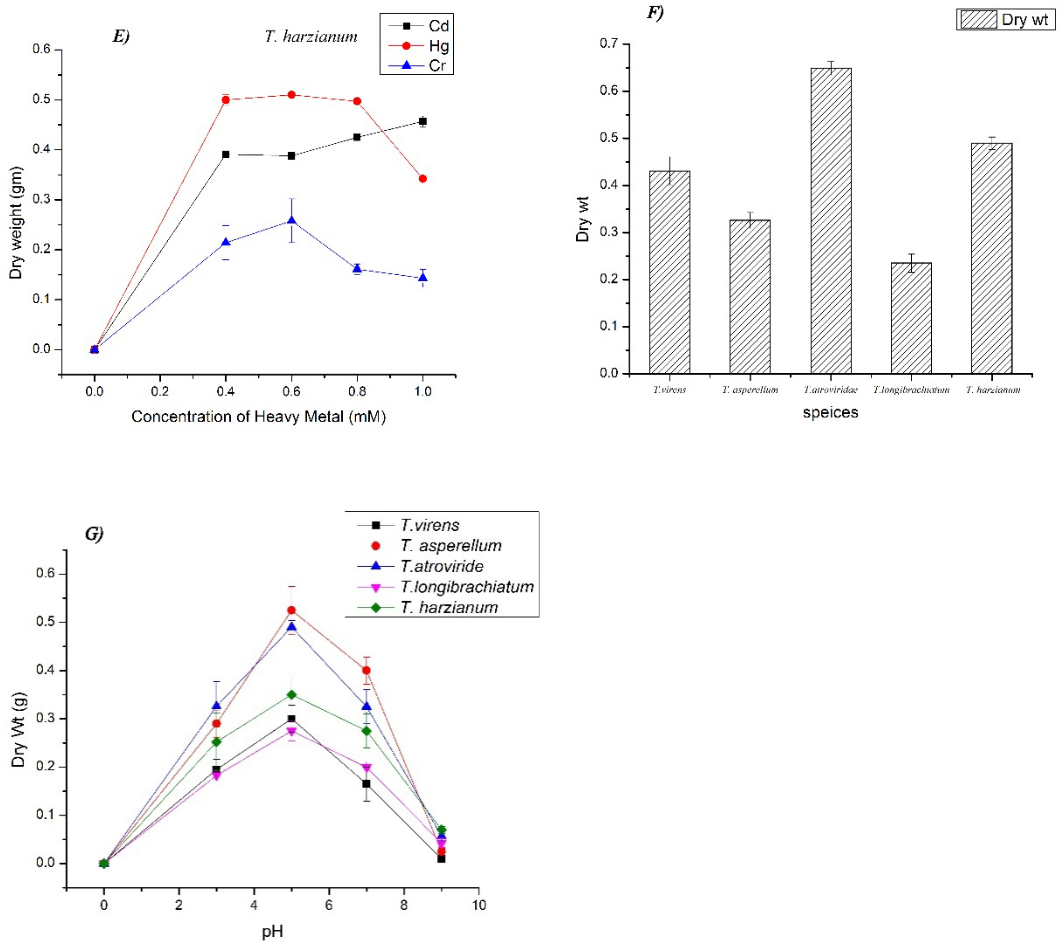


Figure 1: [A] *Trichoderma virens* [B] *Trichoderma asperellum* [C] *Trichoderma atroviride* [D] *Trichoderma longibrachiatum* [E] *Trichoderma harzianum* are the graphical representation of minimal inhibitory concentration different *Trichoderma* strains against Cadmium, Mercury and Chromium salts amended in PDB broth whereas [F] showing of optimal growth rate of all the tested *Trichoderma* strains in PDB without metal salts, [G] Isolates showing optimal growth rate at different pH.

4. Conclusion

In our previous reports with the same rhizospheric soil sample, we have explored the tolerability of bacterial strains against multi-metal ions [8]. In this study, adsorption of metal salts on *Trichoderma* sp. biomass has been investigated and data obtained through this work supports that *Trichoderma* biomass is an effective biosorbent for removal of metal ions. The colonising ability of *Trichoderma* sp. has shown to enhance the interest towards investigating the biochemical abilities. Hence, in future, *Trichoderma* species can be involved in clean-up of heavy metal contaminated sites.

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6. References

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