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Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites

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ABSTRACT

Increased environmental pollution has necessitated the need for eco-friendly clean-up strategies. Filamentous fungal species from gold and gemstone mine site soils were isolated, identified and assessed for their tolerance to varied heavy metal concentrations of cadmium (Cd), copper (Cu), lead (Pb), arsenic (As) and iron (Fe). The identities of the fungal strains were determined based on the internal transcribed spacer 1 and 2 (ITS 1 and ITS 2) regions. Mycelia growth of the fungal strains were subjected to a range of (0–100 Cd), (0–1000 Cu), (0–400 Pb), (0–500 As) and (0–800 Fe) concentrations ($mgkg^{-1}$) incorporated into malt extract agar (MEA) in triplicates. Fungal radial growths were recorded every three days over a 13-days' incubation period. Fungal strains were identified as *Fomitopsis meliae*, *Trichoderma ghanense* and *Rhizopus microsporus*. All test fungal exhibited tolerance to Cu, Pb, and Fe at all test concentrations (400–1000 $mgkg^{-1}$), not differing significantly ($p > 0.05$) from the controls and with tolerance index >1. *T. ghanense* and *R. microsporus* demonstrated exceptional capacity for Cd and As concentrations, while showing no significant ($p > 0.05$) difference compared to the controls and with a tolerance index >1 at 25 $mgkg^{-1}$ Cd and 125 $mgkg^{-1}$ As. Remarkably, these fungal strains showed tolerance to metal concentrations exceeding globally permissible limits for contaminated soils. It is envisaged that this metal tolerance trait exhibited by these fungal strains may indicate their potentials as effective agents for bioremediative clean-up of heavy metal polluted environments.

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Introduction

Increased heavy metal contamination of soil and water environments[1] has necessitated the need for clean-up strategies. Recently, diverse eco-friendly remediation options have been explored for the restoration of contaminated environments. These remediation options, among others, include the use of plants (phytoremediation),[2] bacteria (bacterial bioremediation)[3] and fungi (mycoremediation).[4] The employability of these bio-resources (plants, bacteria and fungi) for effective bioremediation has been well reported.[2–4]

At present of these options, mycoremediation strategy has received increased attention in the bioremediation of contaminated/polluted environments due to its reasonably low cost implications and significant success outcomes.[5–8] Filamentous fungal species have been identified for their distinct attributes (ability to thrive under extreme pH, temperature and nutrient variability conditions, as well as tolerance to high metal concentrations)[9–11] and hence their effective remediation traits of contaminated sites.

Metal tolerance/resistance has been defined as the ability of an organism to survive metal toxicity by means of one or more mechanisms devised in direct response to the metal(s) concerned.[7,12] Metal tolerance by filamentous fungi has been associated with their sites of isolation, toxicity of the metal tested, its concentration in medium, and on the isolate's competence.[10] Contaminated sites are known as principal sources of metal-resistant species[18–22] with indigenous fungal strains isolated from heavy metal contaminated sites exhibiting notable tolerance for high heavy metal concentrations.[9,21,23–25]

However, of more importance is the specific and non-specific heavy metal tolerance mechanisms adopted by fungal species. According to Vadkertiova and Slavikova[13] the introduction of heavy metals into the environment has induced physiological and morphological adaptation strategies in the microbial community. Specifically, fungal species adopt one or more metal tolerance strategies which include extracellular metal sequestration and precipitation, suppressed influx, enhanced metal efflux, production of intracellular/extracellular enzymes, metal binding to cell walls, intracellular sequestration and complexation.[14–17]

Several metal-tolerant filamentous fungi (*Rhizopus*, *Trichoderma*, *Aspergillus*, *Penicillium*, and *Fusarium*) have been isolated from multiple heavy metal contaminated soils.[7] Zafar et al.[7] reported that *Rhizopus* sp., isolated from metal-contaminated agricultural soils tolerated Cd and Cr concentrations. In addition, Volesky[26] observed that the mycelium of a *Rhizopus* specie was biosorbent towards Pb, Cd, Cu and Zn. *Trichoderma* species have also been known to exhibit tolerance to a range of toxicants[27–29] and Cu, Cd, As and Zn heavy metals in in vitro conditions.[8,23,27,30–34]

However, there is a dearth of knowledge of the growth response and heavy metal tolerance of filamentous fungal species isolated from gold and gemstone mining sites. This study was therefore designed to isolate, identify and assess the growth response and tolerance/resistance of filamentous fungi isolated from gold and gemstone mining sites to varied concentrations of selected heavy metals associated with mining sites.

Materials and methods

Study sites and soil sampling

Mine site soils used in this study were obtained from gemstone and gold mining sites in Southwestern, Nigeria namely: Awo (7°46′ N, 4°24′ E) and Itagunmodi (7°30′ N, 4°49′ E) as described.[1,4,35] From previous studies,[1,4,35] soil preliminary heavy metal analysis of the sites recorded elevated concentrations of 0.20–0.35 $mgkg^{-1}$ Cd; 3.68–48.60 $mgkg^{-1}$ Cu; 19.05–35.00 $mgkg^{-1}$ Pb; 20.45–34.80 $mgkg^{-1}$ As and 240.24–296.18 $mgkg^{-1}$ Fe.

Isolation of soil fungi

Isolation of soil fungi was performed by serial dilution and the spread plate method using malt extract agar (MEA) medium and incubated at 30°C for five days as previously described.[4,35] Streptomycin (35 $mgmL^{-1}$) was added as a supplement into the medium to inhibit bacterial growth. After incubation, isolates of single spores were successively sub-cultured on MEA to obtain pure isolates. Fungal species were characterized on the basis of phenotypical/macroscopic observation (pigmentation, shape, diameter, colony appearance and texture) and microscopic examination (septation of mycelium, shape, form, diameter and texture of spore/conidia). The cultural and morphological characteristic features of the fungal species were compared with those described.[36] Fungal species were then selected for genotypic-based identification.

DNA extraction and PCR amplification

The ZR fungal/bacterial DNA kit (Zymo Research, Irvine, CA, USA) was used to extract genomic DNA from pure 5-day old fungal cultures according to the manufacturer's manual. About 40 mg (wet wt.) mycelium was harvested aseptically into the ZR BashingBead™ lysis tube and lysed in 750 μL of lysis buffer by bead beating. The lysate was then centrifuged at 13,400 rpm for 300 s to obtain clear supernatant. Further protocols, which are binding, wash steps and elution of DNA were performed as instructed by the manufacturer. The quality and integrity of the extracted DNA were verified on 1% agarose gel, while DNA concentration and purity were verified using NanoDrop spectrophotometer (ND-1000, NanoDrop Technologies Inc., Wilmington, Delaware USA).

Taxonomic identification of isolates was between the internally transcribed spacer regions – 1 (ITS1) and 2 (ITS2). DNA amplification was done using primer sets ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′).[37] Each PCR reaction contained 12.5 μL of 2× Dream Master mix (Thermo Scientific Technologies, Waltham, MA, USA), 50 ng DNA template, 0.2 M of each forward and reverse primers and nuclease-free water to a final volume of 25 μL. PCR was performed in a C1000™ thermal cycler (Bio-Rad, Hercules, CA, USA) involving an initial denaturation at 95°C for 5 min, 29 cycles of denaturation at