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Isolation of Endophytic Fungi from *Tridax Procumbens* Grown in Contaminated Sites and Assessment of Their Heavy Metal Tolerance

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Abstract: Seven strains of endophytic fungi were isolated from the leaves of *Tridax procumbens* and were first screened individually for tolerance to heavy metals like Zn, Pb, Cu, Mg, and Cr from 5 ppm to 50 ppm on Potato dextrose agar medium. They were further tested for resistance at 75, 100, 200, 400, and 600 ppm of heavy metals. The fungal isolate on the PDA medium without any heavy metal was used as a control for growth comparison. It was observed that with increasing concentration of heavy metal from 5 to 600 ppm there was a decrease in the number of fungal isolates. Few of the fungal isolates were able to tolerate heavy metals up to 600 ppm. The screening test revealed heterogeneity in the heavy metal tolerance of the isolates.

Keywords: Heavy metal tolerance, endophytic fungi, *Tridax procumbens*, metal pollution

Introduction

Heavy metals are high density metals that are toxic even at ppb levels. Heavy metal pollution is a major problem in the environment all over the world. Heavy metals are continuously discharged into the environment due to various industrial activities. Heavy metals like Cd, Cr, Co, Cu, Pb, Ni, Hg, and Zn are considered as the most hazardous metals by the World Health Organization (WHO) [1]. A soil with the natural composition of heavy metal does not affect animals or plants. Heavy metals like Zn, Pb, Cu, Mg, and Cr when released above certain threshold levels can contaminate and be toxic to the soil, surface water, groundwater and sediments [2] and can exert certain deleterious effects on the biological system. They cannot undergo biodegradation and are hence in living organisms, leading to various diseases and disorders even at low concentrations.

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These metals cause damage to brain, can lead to other effects like reproductive failures, nervous system failures, and can also induce tumour formation in human beings [3].

Heavy metal pollution can be studied by the estimation of the total number of tolerant microorganisms. The bacteria and fungi isolated from polluted environments showed high-level tolerance to metals when compared to bacteria and fungi isolated from unpolluted environments [4]. Bioremediation of heavy metals can be best attained by the use of endophytic organisms. Fungal endophytes live in a mutualistic relationship with plant host for at least a part of their life, without causing any disease. The resistance of endophytic fungi to heavy metals and their efficiency for the removal of heavy metals differs greatly. *T. procumbens* is a widespread weed seen throughout the year in India [5] which removes effectively heavy metals like Cr, Cu, Ni, Pb, and Cd from soil contamination [6]. *T. procumbens* belongs to Asteraceae family and is a perennial plant. This species found usage in ayurvedic medicine in ancient times. [7]. It is common in tropical or semitropical climate fields, croplands, lawns, and roadsides [8]. Several approaches are used for the reduction of heavy metal pollution which are found ineffective due to incomplete removal of heavy metals. Hence the investigation was aimed to isolate endophytic fungi from *T. procumbens* and to screen their metal tolerance and resistance level to few heavy metals under lab conditions.

Materials and methods

Collection of sample:

Five different *Tridax procumbens* samples were collected from different industrial polluted sites in Chennai city (Fig.1(a), 1(b)). The plants were carefully uprooted and collected using polythene bags. The confirmation of the plant was done at the Department of Plant Biology and Biotechnology Quaid-e-millath Government College, Chennai. The leaves of *T. procumbens* were separated were washed in running tap water.

Fig. 1(a) and Fig.1(b): *T. procumbens* were collected from different industrial polluted sites.

Surface sterilization:

The leaves were washed using sterile distilled water for 2 to 3 times and rinsed thoroughly with 75% ethanol (Fig.2a). After surface sterilization, the leaves were air-dried in sterile conditions (Fig.2b).

Fig.2(a)

Fig.2(b)

Fig.2(c)

Fig.2 (a, b & c): Surface sterilized *T. procumbens* and leaves plated in PDA

Isolation and Identification of Endophytic Fungi:

Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol was used as a culture medium. The freshly prepared medium was sterilized and under aseptic conditions was poured to sterile petriplates and allowed to cool. The surface sterilized leaves of *T. procumbens* were placed on to sterile PDA plates (Fig.2c) and, incubated at 25°C for 4 to 6 days. The mycelia of dominant isolates were transferred to a fresh PDA medium to obtain pure fungi strains. Pure isolates of endophytic fungi were characterized by the macroscopic appearance and microscopic appearance.

Heavy metal resistant endophytic fungal selection:

Isolated endophytic fungi were screened individually for heavy metal tolerance at concentrations from 5 to 50 ppm to Zinc chloride (Zn), Lead acetate (Pb), Cupric sulphate (Cu), Magnesium chloride (Mg), and Potassium dichromate (Cr). PDA medium inoculated with the isolate without any heavy metal is served as the control for comparison of fungal growth. Observations were recorded in comparison to control as presence or absence of growth. Heavy metal-tolerant (50ppm) fungal isolates were further tested for tolerance to increasing concentrations of 75, 100, 200, 400, and 600 ppm of Zn, Pb, Cu, Mg, and Cr individually on PDA. The plates were inoculated with young fungal culture and it was incubated at 25°C for 7-14 days [9], [10].

Results

Isolation and identification of endophytic fungi:

Seven dominant endophytic fungi were successfully isolated from the leaves of *T. procumbens* collected from different industrial polluted sites. Preliminary identification based on their morphological characteristics on PDA and microscopic examination of the isolates after staining with Lactophenol cotton blue (LPCB) revealed the isolates could be related to the following genera *Fusarium* sp. (strain 1 - *Fusarium* sp. (Fig.3.1), strain 2 - *Fusarium* sp. (Fig.3.2), *Aspergillus* sp. (strain 3 - *A. flavus* (Fig.3.3), strain 4 - *A. niger* (Fig.3.4), strain 5 - *Curvularia* (Fig.3.5), strain 6 and strain 7 (Fig.3.6 & 3.7).

Screening and determination of tolerance levels of endophytic fungal isolates to Zn, Pd, Cu, Mg, and Cr:

The endophytic fungal isolates from *T. procumbens* collected from different industrial polluted sites were screened for tolerance from 5 ppm to 600 ppm concentration of each heavy metal (Zn, Pb, Cu, Mg, and Cr). All the isolates were tolerant of all the heavy metals at varying concentrations. Maximum tolerance was shown towards Mg by all isolated strains at 600 ppm (table.1). Strain 1 & 3 showed high tolerance to Zn at 600 ppm and strain 7 (400ppm), strain 2 (200ppm), strain 5 and 6 (100ppm). Strain 4 (50ppm) showed the lowest tolerance for Zn. Maximum tolerant to Cu was showed by strain 3 at (600ppm), strain 1 and 5 (400ppm), strain 4 (200ppm), strain 2, 6 and 7 (100ppm). The highest tolerance to Pb was shown by strain 1, 3 and 4 at (600ppm), strain 5 and 7 (400ppm), and strain 2 and 6 (200ppm). Cr showed the strongest inhibition of growth. Strain 1, 3, 5 and 7, could tolerate a concentration of 50 ppm, and other strains to a lesser concentration strain 6 (25ppm), strain 2 (10ppm) & strain 4 (10ppm) shows minimum tolerance.

The ranges of heavy metal tolerance of all the fungal isolates are presented in Table 1. It was observed that with the increase in the concentration of heavy metal there was a decrease in the growth of the isolate. The heavy metal tolerance limits of fungal isolates are presented in (Fig. 4).

Fig. 3: Microscopic examination of isolated endophytic fungi

Fig.3.1 (a, b) *Fusarium* sp.

Fig.3.2 (a, b) *Fusarium* sp.

Fig.3.3 (a, b) *A. flavus*

Fig.3.4 (a, b) *A. niger*

Fig.3.5 (a, b) *Curvularia*

Fig.3.6 (a, b) S6

Fig.3.7 (a, b) S7

Table 1: Tolerance indices of Endophytic fungal isolates to heavy metals Zn, Pb, Cu, Mg, and Cr.

Strains	Zn	Pb	Cu	Mg	Cr
S1	600 ppm	600 ppm	400 ppm	600 ppm	50 ppm
S2	200 ppm	200 ppm	100 ppm	600 ppm	10 ppm
S3	600 ppm	600 ppm	600 ppm	600 ppm	50 ppm
S4	50 ppm	600 ppm	200 ppm	600 ppm	10 ppm
S5	100 ppm	400 ppm	400 ppm	600 ppm	50 ppm
S6	100 ppm	200 ppm	100 ppm	600 ppm	25 ppm
S7	400 ppm	400 ppm	100 ppm	600 ppm	50 ppm

GRAPH.1: Tolerance indices of isolated strains to various heavy metals Zn, Pb, Cu, Mg, and Cr. **GRAPH.1(a)** **GRAPH.1(b)** **GRAPH.1(c)** **GRAPH.1(d)**

GRAPH.1(e)

GRAPH.1(f)

GRAPH.1(g)

Fig.4.1: Growth of S1 (*Fusarium sp.*) after exposure to different concentrations of heavy metal ions for 7-14 days.

Fig.4.2: Growth of S2 (*Fusarium sp.*) after exposure to different concentrations of heavy metal ions for 7-14 days.

Fig.4.3: Growth of S3 (*A. flavus*) after exposure to different concentrations of heavy metal ions for 7-14 days.

Fig.4.4: Growth of S4 (*A. niger*) after exposure to different concentrations of heavy metal ions for 7-14 days.

Fig.4.5: Growth of S5 (*Curvularia*) after exposure to different concentrations of heavy metal ions for 7-14 days.

Fig.4.6: Growth of S6 after exposure to different concentrations of heavy metal ions for 7-14 days.

Fig.4.7: Growth of S7 after exposure to different concentrations of heavy metal ions for 7-14 days.

Discussion

Contaminated sites are major sources of heavy metal resistant microorganisms. Due to prolonged exposure of plants to heavy metals there are modifications in their microbial populations. Heavy metal pollution of the soil leads to a decrease in microbial diversity. This is because some species that are sensitive to the imposed stress and due to the enhanced growth of other resistant species are unable to survive and become extinct gradually.

Due to the metal binding properties of their cell wall materials, Fungi are known to tolerate heavy metals readily [4]. Endophytes acquire tolerance and heavy metal resisting ability from the host as they live in close relation to the host. Hence the study was aimed to isolate heavy metal tolerant endophytic fungi from a plant grown in polluted sites.

The endophytic fungi were isolated from leaves of *T. procumbens* grown on industrial polluted site which is polluted with heavy metals and other pollutants in the soil for several years. Fungi isolated as per previous International taxonomic keys belonged to the genera *Fusarium*, *Aspergillus* sp. (*Aspergillus flavus*, *A. niger*), *Curvularia*, and unidentified isolates 6 & 7. Further confirmation of the strains should be made by sequencing analysis. All the isolates were screened for tolerance against zinc, lead, copper, magnesium, and potassium dichromate starting at a concentration of 5 ppm to 600 ppm. *Aspergillus* sp and *Fusarium* sp. could tolerate lead concentration of 600 ppm. *Curvularia* sp and Isolate 7 could tolerate to a lesser degree with MIC value of 400 ppm.

Cr showed the strongest inhibition towards all the isolates at a concentration of 50 ppm. The majority of the tested fungi were unable to grow in the presence of this metal and were sensitive to the presence of this metal ions in the growth. Zinc at a concentration of 600 ppm was tolerated by *Aspergillus* sp and at 400 ppm by strain 7 and to a lesser degree by other isolates. Copper at a concentration of 400 ppm was tolerated by *Fusarium* sp and to a lesser concentration of 200 ppm by other isolates.

The screening test showed diversity in the heavy metal tolerance of the isolates. Few researchers have reported similar results [4], [11]. There were variations in resistance against individual metals among the isolates. The results indicate that the resistance level against individual metals was dependent on the isolates and different species of fungus showed different tolerance pattern. Some were sensitive, moderately tolerant, and tolerant and varied with different metals. Isolates of the same genus also showed a considerable difference in metal resistance level.

Noticeable differences in Cu, Zn, and Cr tolerance have also been found among the isolates. This differences in metal tolerance shown by different isolates may be due to difference in tolerance processes and resistance mechanisms. Few isolates of *Aspergillus flavus* were more tolerant for both metals (Cr & Pb) while others were very sensitive to Cr but tolerant to Pb.

Conclusion

As the heavy metals have toxic effects on human, animal, and plant health. It is essential to minimize heavy metal concentrations in the environment. Microbial remediation or

bioremediation has the advantages of the removal of large amounts of heavy metal efficiently at a low cost. Endophytic fungi show excellent metal-binding capacity and have the ability to protect the host against heavy metal toxicity. Hence in this study endophytic fungi from the leaves of *Tridax procumbens* collected from five different industrial polluted sites were tested for their tolerance and ability to remove heavy metals from the solid medium in lab condition. The isolated fungus showed appreciable potential to grow in the presence of Cr, Zn, Pb, Cu, and Mg and some isolates could tolerate heavy metals up to 600 ppm. Further investigations are needed to confirm the isolates.

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Conflict of interest: None

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