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Isolation and Identification of *Trichoderma* in Soil Samples from Waste Dumping Sites, Matara, Sri Lanka

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Abstract- Urban waste dumping sites pose significant challenges to environmental health and sustainability. In this study, soil samples were collected from eight waste dumping sites in Matara district, Sri Lanka, to investigate the presence and diversity of fungal species. Fungal species were isolated using Potato Dextrose Agar medium and characterised based on morphological and molecular analyses. Molecular identification was performed by PCR amplification of the internal transcribed spacer (ITS) region of the rDNA gene. Further, these species' production of cellulase and amylase was assessed using plate-based methods. Microscopic and macroscopic features, including colony morphology, conidial structures, and growth patterns, revealed the presence of different *Trichoderma* species in the soil samples. In molecular biological assays, preliminary results indicated two *Trichoderma* species, namely *Trichoderma lixii* (PP086873) and *Trichoderma harzianum* (PP092041), suggesting the adaptability of these fungi to these challenging environmental conditions. Both isolates showed rich depolymerizing enzymatic profiles. The findings of this study contribute to the understanding of fungal diversity in urban waste environments and serve as a foundation for devising strategies to augment the waste degradation capabilities of these fungi. Addressing potential limitations is essential to ensure the reliability and validity of applying the findings beyond the specific location and environmental conditions.

Keywords: Isolation, identificación, *Trichoderma*, waste dumping sites

Suggested Citation

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1. Introduction

Fungus *Trichoderma*, found in soil ecosystems, possess notable properties for agricultural use. They efficiently colonize plant roots and exhibit biocontrol potential against diverse soil phytopathogens through mechanisms like mycoparasitism and competition for nutrients. Their multifaceted approach, including the degradation of pathogen cell walls and induction of plant resistance, positions them as valuable alternatives to chemical pesticides. Its non-pathogenic nature and ability to stimulate plant growth through biofertilization and the production of phytohormones make it an appealing candidate for green technologies in agriculture (Tyśkiewicz *et al*., 2022).

While *Trichoderma* species are not traditionally recognized as primary agents for urban waste degradation, their versatile characteristics, such asmycoparasitism and production of various hydrolytic enzymes, have led to increased interest in their potential role in waste management. Recent studies suggest that certain *Trichoderma* strains may contribute to the breakdown of organic components in urban waste, particularly lignocellulosic materials. By secreting enzymes like cellulases and hemicellulases, *Trichoderma* can facilitate the decomposition of complex organic structures, potentially aiding in converting urban waste into simpler, more environmentally benign compounds (Organo *et al*., 2022). However, applying *Trichoderma* for urban waste degradation is an evolving area of research and practical implementation would require further investigation into the specific waste types and conditions that optimize its effectiveness in the complex urban waste environment.

Biological agents and their metabolic enzymes can be exploited as potent tools for waste biodegradation. The ability of fungi in waste biodegradation is enhanced by producing these enzymes (Karigar & Rao, 2011; Khruengsai *et al*., 2021). Further, in a study on biodegradation plastic waste, authors have reported that fungi secrete different sets of enzymes depending on the molecular weight of the waste substrate (Nowak *et al*., 2012).

The study was designed to isolate abundant fungal species from soil samples in urban waste dumping sites in the Matara district of Sri Lanka. Overall, the results demonstrated the predominance of *T. lixii* and *T. harzianum* in the selected locality. The diversity and distribution of *Trichoderma* species in the sampled sites were analysed to understand their potential role in waste decomposition and soil health. This research provides valuable insights into the ecology of *Trichoderma* species in urban waste environments and their potential role in sustainable agricultural practices. Further investigations into the functional aspects of these fungi and their interactions with other soil microorganisms will enhance the understanding of their ecological significance.

2. Materials and Method

Soil samples were collected from eight locations in urban waste dumping sites in the Matara district to isolate fungi. The locations were almost flat and had no complex topographic features. Those were Dondra dumping site (5°55'55.35"N, 80°35'27.81"E), Weligama dumping site (5°58'38.56"N, 80°25'43.86"E), Thalalla dumping site (5°56'32.16"N, 80°37'0.24"E), Walpala dumping site (5°57'21.98"N, 80°32'52.84"E), Walgama dumping site (5°56'46.94"N, 80°30'47.82"E), Mirissa dumping site (5°56'53.74"N, 80°28'17.71"E), dumping site near Nilwala river basin (5°56'45.58"N, 80°32'54.09"E) and dumping site near Dikwella lagoon (5°58'18.03"N, 80°41'42.37"E).

The soil sampling process followed a previously established method (Ameh & Kawo, 2017). Loose, unconsolidated surface soil was used to attain composite samples. The samples were carried to the laboratory in a sterilized container inside an ice pack. The soil samples were passed through a 4 mm sterilized sieve. Samples were placed at refrigerated conditions (4 °C) until soil suspension was prepared for fungal isolation.

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The suspension was prepared with a few modifications to the method described previously (Ameh & Kawo, 2017). The soil sample (1.00 g) was added to sterilized water (9.0 mL), followed by homogenization. Samples were pour-plated with Potato Dextrose Agar (PDA). The plates were incubated at room temperature (28 ± 2 °C) for 3-5 days. All soil samples were analyzed in duplicates, and the number of colonies was counted. For fungal isolation, hyphal tips from morphologically distinct colonies were placed on PDA plates and subjected to several subcultures to obtain pure cultures. Plates were incubated at room temperature (28 ± 2 °C) for 3-5 days. The isolated fungal colonies were stored on PDA slants and kept at 4 °C for further characterization and identification.

The fungi were identified based on their colony characteristics, morphology of hyphae and spores. Microscopic analysis was done using the Lacto Phenol Cotton Blue staining method (McClenny, 2005). In fungal species identification, the nuclear rDNA ITS region was amplified using fungal-specific ITS1 forward, and ITS4 reverse universal primers (White *et al*.,1990). The amplified PCR products were sequenced using Sanger dideoxy sequencing technology at Genetech Institute, Colombo, Sri Lanka. Sequences were manually edited using BioEdit sequence Alignment Editor (Version 7.2.5) and were compared with the sequences available in the GenBank using BLAST to assess homology. DNA sequences of the identified fungal species were deposited in the NCBI database, and accession numbers were obtained. Qualitative cellulase activity was assessed by growing the pure isolates on 1% carboxymethyl cellulose (CMC) medium and spilling 1% congo red after incubation to test its cellulolytic potential (Béguin, 1983). In qualitative amylolytic assays, isolates were inoculated in starch agar plates. Plates were incubated at 28 ± 2°C for 5 days and were flooded with 1% KI/I_2 solution (Kannangara *et al*.,2009; Perera *et al*.,2022).

3. Results and Discussion

This study focused on the isolation and identification of fungal species from soil samples collected at eight urban waste dumping sites. The analysis revealed a diverse fungal flora, with a particular emphasis on the presence and identification of *Trichoderma* species.

As indicated in Table 1. quantitative analysis revealed variations in the colony-forming units (CFUs) of fungal species among the different sites. The Dondra dumping site exhibited the highest CFU count, with an average of 8.0×10^4 CFU/g of soil, while the Dikwella lagoon site had the lowest count, averaging 2.5×10^3 CFU/g. The highest abundance of fungal species was found in samples from the Dondra dumping site and Weligama site, which had more favourable conditions for fungal growth due to the acidic soil pH.

Table 1

The results of the mean fungal counts of eight locations.

Location	pH	Mean fungal counts (CFU g^{-1})
Dondra dumping site	4.45	8.0×10^4
Weligama dumping site	5.60	6.0×10^4
Thalalla dumping site	6.05	1.1×10^4
Walpala dumping site	6.90	9.9×10^3
Walgama dumping site	7.15	8.5×10^3

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Mirissa dumping site	7.95	5.7×10^3
Dumping site near Nilwala river basin	8.20	3.3×10^3
Dumping site near Dikwella lagoon	8.95	2.5×10^3

In the present study, a total of two morphologically different fungal isolates were commonly and predominantly detected in all soil samples (Figure 1). The author successfully isolated these two key species into pure cultures, and isolates were subjected to fungal identification assays using morphological approaches. Eventually, species confirmation was achieved by comparing gene sequences. The identification through ITS sequencing confirms the reliability of combining morphological and molecular techniques for fungal identification.

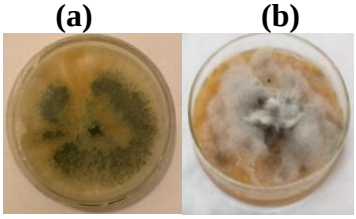

Figure 1. *Isolated fungal colonies grown in pure cultures on Potato Dextrose Agar* ***(a)*** *KLN-N-001* ***(b)*** *KLN-N-004.*

The identities of the two fungal isolates were revealed in the current study as *Trichoderma harzianum* (PP092041) and *T. lixii* (PP086873). They were the more frequently isolated species, indicating their widespread presence and potential ecological significance in these waste dump environments. The presence of *Trichoderma* species across all sampled sites reinforces the role of these fungi in waste decomposition and nutrient cycling within urban waste dump ecosystems.

Among the isolates, the particular strain, designated KLN-N-001, exhibited distinctive morphological and genetic characteristics. The fungal isolate KLN-N-001 demonstrated a floppy growth pattern with green conidial production when cultured on PDA. Microscopic examination revealed that the conidia were globose, and the phialides were flask-shaped (Figure 2a). Genetic analysis further confirmed the identity of KLN-N-001. The ITS sequence of the strain was compared with sequences in the GenBank database. The ITS sequence of KLN-N-001 matched with the *Trichoderma harzianum* (MT605289) sequence, showing a sequence similarity of 98.48%. This high level of similarity confirms that KLN-N-001 is a strain of *T. harzianum*, a member of the division Ascomycota. Notably, the closest match for this strain was previously isolated from soil samples in the Black Sea Region of Turkey, indicating a broad geographic distribution for this species (Kushiyev *et al*.,2021) The discovery of KLN-N-001 in Sri Lanka, with its closest genetic match from Turkey, suggests the wide ecological range and adaptability of *T. harzianum*. The isolated strain KLN-N-001 exhibited significant enzymatic activities, which are critical for its role in organic matter degradation. These enzymatic properties highlight the potential application of *T. harzianum* in bioremediation and waste management.

The fungal isolate KLN-N-004 initially exhibited white cottony hyphae with a smooth and watery appearance. Over time, the mycelium developed a fuzzy, fluffy aerial structure. Microscopic examination revealed pyramid-shaped conidiophores and scattered conidia. The flask-shaped phialides bore globose conidia (Figure 2b). The ITS sequence of the strain matched with the *Trichoderma lixii* (OR116249) sequence from GenBank, showing a sequence similarity of 100.00%. This perfect match verifies that KLN-N-004 is a strain of *T. lixii*, a

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member of the division Ascomycota. Its isolation evidence was available from the northwestern Himalayas for the production of bioactive natural products (Katoch *et al*.,2019). The isolation of *T. lixii* from multiple urban waste dumping sites in the Matara district underscores the ecological versatility and adaptability of this species.

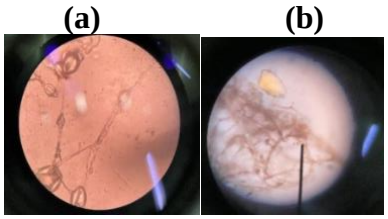

Figure 2. *Fungal identification using morphological techniques under the optical microscope **(a)** KLN-N-001 **(b)** KLN-N-004.*

Both isolates, KLN-N-001 and KLN-N-004, exhibited significant enzymatic activities critical for their role in organic matter degradation (Figure 3). *Trichoderma harzianum* displayed high cellulase activity, making it an excellent candidate for enhancing cellulose degradation in waste management processes. Similarly, both isolates showed amylase activity, which is beneficial for starch decomposition. The findings from this study suggest that both species can be harnessed to improve the efficiency of organic waste degradation. The diversity and abundance of *Trichoderma* species observed in this study align with findings from similar environments, although the specific composition varied slightly (Manczinger *et al*.,2002; Hoyos-Carvajal *et al*.,2009; Dou *et al*.,2019).

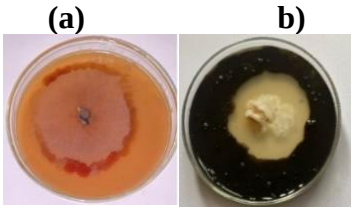

Figure 3. *Enzymatic assays using plate-based methods for the fungal isolates **(a)** cellulase production **(b)** amylase production.*

Applying spore suspensions of these strains could enhance the breakdown of organic materials in waste dumps, leading to more sustainable waste management practices. Further research should focus on optimizing the conditions for spore application and assessing the long-term impacts on waste decomposition rates.

This study contributes to a better understanding of fungal communities in waste environments and their role in organic matter degradation by focusing on the isolation, identification, and analysis of *Trichoderma* species from urban waste dumping sites. Applying *Trichoderma* species in bioremediation efforts could present a promising avenue for enhancing the efficiency of waste decomposition and promoting sustainable waste management. Further research and identification of these fungi may contribute to a more nuanced understanding of their roles and impacts in these unique ecosystems.

4. Conclusion

The study demonstrated the dispersion of *Trichoderma* species in urban waste dumping sites within the selected locations in the Matara district, and in all the tested soil samples, the predominance of *T. lixii* and *T. harzianum* was confirmed. The findings would serve as a

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baseline for the future expansion of the study in applying these fungi to the urban waste degradation processes.

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