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ISOLATION AND MOLECULAR IDENTIFICATION OF SOIL-DERIVED FUNGI WITH AROMA-PRODUCING POTENTIAL

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ABSTRACT

Background: Soil-derived fungi represent an important source of bioactive metabolites and volatile compounds with potential applications in the flavour and fragrance industries. *Trichoderma* species are known for their diverse metabolic capabilities, including the production of volatile organic compounds (VOCs). The present study aimed to isolate soil-derived fungi, screen them for aroma production, and identify the selected aroma-producing isolate using molecular methods. **Methods:** Soil samples from Gautala Abhayaranya, Maharashtra, India, were subjected to serial dilution and cultured on PDA. Twenty fungal isolates were obtained and screened for characteristic aroma, and one aroma-producing isolate was selected for molecular identification. The ITS region was amplified using ITS1/ITS4 primers, sequenced, and compared with UNITE database sequences using BLAST. Phylogenetic analysis was performed using the maximum-likelihood method. **Results:** Among the 20 fungal isolates obtained, one isolate exhibited a distinct characteristic aroma and was selected for further analysis. ITS sequence analysis showed the highest similarity of the isolate to *Trichoderma nordicum*, with 99.826% sequence identity and 100% query coverage. Phylogenetic analysis further placed the isolate in close association with *T. nordicum*, supporting its molecular identification. **Conclusion:** The study resulted in the isolation and molecular identification of an aroma-producing *T. nordicum* isolate from soil. The characteristic aroma exhibited by the isolate indicates its potential as a source of volatile metabolites of flavour and fragrance interest. Further studies involving GC-MS analysis are required to identify and characterise the volatile compounds responsible for the observed aroma and to evaluate their potential applications.

KEYWORDS: Soil-derived fungi, Molecular Identification, Aroma-Producing Potential.

INTRODUCTION

The genus *Trichoderma* comprises filamentous fungi belongs to the phylum Ascomycota and is widely distributed in different environments, particularly soil, plant tissues, water, and decaying organic matter. Species of *Trichoderma* are known for their ability to produce a wide variety of extracellular enzymes and secondary metabolites with diverse biological activities. These metabolites play important roles in the antagonistic activity of *Trichoderma* against plant pathogens through mechanisms such as mycoparasitism, antibiosis, competition, and induction of plant defence responses.[1,2]

In addition to non-volatile metabolites, *Trichoderma* species produce a diverse range of volatile organic compounds (VOCs) that interact with plants and other microorganisms. Several VOCs produced by *Trichoderma* have been associated with antimicrobial activity and plant growth-promoting effects.[3] Some of these volatile metabolites are also of interest for their characteristic odours and potential applications in fragrance production. Among the reported compounds, 6-pentyl-α-pyrone (6-PP) is one of the most extensively studied aroma compounds produced by *Trichoderma* and is characterized by a pleasant coconut-like aroma.[4] The type and quantity of VOCs produced may differ among species and strains, and are also influenced by the growth substrate and culture conditions.[4,5]

Therefore, the isolation and identification of *Trichoderma* strains from different ecological sources may provide an opportunity to explore strains with potentially valuable metabolic characteristics, including the production of aroma-associated volatile compounds.

MATERIAL AND METHODS

Collection of the soil samples

Soil samples were collected from different sites within Gautala Abhayaranya, located in Kannad Taluka, Chhatrapati Sambhajinagar District, Maharashtra, India (20.332500° N, 75.140833° E). Samples were collected from selected locations within the forest area and transferred aseptically into sterile plastic bags. The collected samples were properly labelled and transported to the Microbiology Laboratory for further isolation and identification of fungal strains.

Isolation of the fungal strains

Soil fungi were isolated using the soil dilution plate method described by Waksman 1927.[6] 1 g of each soil sample was suspended in 9 mL of sterile distilled water to obtain a 10^{-1} dilution. Serial tenfold dilutions were subsequently prepared up to 10^{-4}. Aliquots (1 mL) of the 10^{-3} and 10^{-4} dilutions were transferred onto sterile Petri plates containing Potato Dextrose Agar (PDA). Streptomycin was added to the medium to suppress bacterial growth. The plates were incubated at 28 °C for 4–7 days and examined periodically for fungal growth.[7, 8]

Individual fungal colonies were sub-cultured onto fresh PDA plates to obtain pure cultures. The purified fungal isolates were subsequently subjected to sensory evaluation to identify strains exhibiting characteristic aroma production. Selected aroma-producing isolates were further investigated for identification.[4]

Molecular identification and phylogenetic analysis:

Molecular identification of the selected fungal isolate was carried out by a HiGenoMB genetic identification laboratory. Genomic DNA was extracted from a pure fungal culture using a commercially available DNA extraction kit (HiMedia) according to the manufacturer's instructions. The ITS region was amplified by PCR using the universal fungal primers

ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′).[9] The amplified PCR product was sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3500xL Genetic Analyzer. The obtained ITS sequence was compared with reference sequences in the UNITE database (version 9.0) using BLAST[10] for species-level identification. Phylogenetic analysis was performed using software iqtree Version 2.1.2, and a maximum-likelihood phylogenetic tree was constructed with 1000 bootstrap replicates.[11]

RESULT

Isolation and screening of fungi isolated from soil

In the present study, a total of 20 fungal isolates were obtained from the collected soil samples. Based on sensory evaluation, T1 isolate exhibiting a distinct characteristic aroma was selected for further molecular identification.

Molecular identification

The aroma-producing potential fungal strain isolated from soil was identified as *Trichoderma nordicum.*

Table 1: BLAST-based identification of the selected fungal isolate using the ITS sequence.

Closest reference species	Accession No.	Query coverage (%)	Percentage identity (%)
Trichoderma nordicum	NR_184883.1	100	99.826
Trichoderma atroviride	NR_077207.1	99	99.824
Trichoderma scalesiae	NR_144876.1	100	99.652
Trichoderma neokoningi	NR_138446.1	100	99.303
Trichoderma dorotheae	NR_166014.1	100	99.129
Trichoderma petenseni	NR_138442.1	100	98.955
Trichoderma hispanicum	NR_138451.1	100	98.955
Trichoderma samuelsii	NR_138452.1	100	98.955
Trichoderma koningii	NR_138456.1	100	98.955
Trichoderma caribbaeum var. *caribbaeum*	NR_166015.1	100	98.955

The Table 1 BLAST analysis of the ITS sequence showed high sequence similarity between the selected fungal isolate and several *Trichoderma* species. The highest similarity was observed with *Trichoderma nordicum* (GenBank accession NR_184883.1), showing 99.826% sequence identity with 100% query coverage. The isolate also showed high similarity to *T. atroviride* (99.824%), *T. scalesiae* (99.652%), and other closely related *Trichoderma* species, with sequence identities ranging from 98.955 to 99.824% and query coverage of 99–100%. Based on the highest sequence similarity, the isolate was assigned to *T. nordicum*, and its taxonomic placement was further evaluated through phylogenetic analysis.

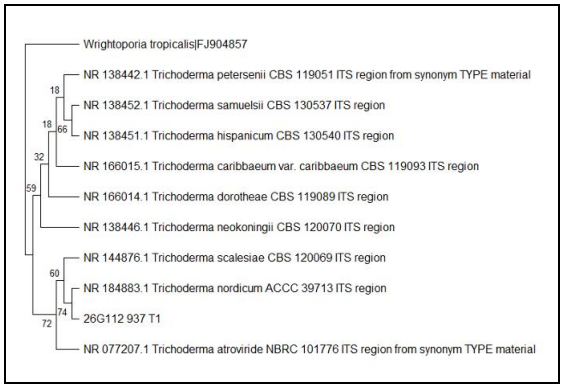

Figure 1: Maximum-likelihood phylogenetic tree based on ITS sequences showing the placement of isolate 26G112_937_T1 among closely related Trichoderma species.

The Figure 1 illustrates that molecular identification of the aroma-producing fungal isolate was performed based on ITS sequence analysis. BLAST analysis showed that the ITS sequence of isolate 26G112_937_T1 exhibited the highest sequence similarity to *Trichoderma nordicum* (NR_184883.1), with 99.826% sequence identity and 100% query coverage. The sequence also showed high similarity to other closely related *Trichoderma* species, including *T. atroviride* (99.824%) and *T. scalesiae* (99.652%). Phylogenetic analysis based on the ITS sequences further supported the identification, with isolate 26G112_937_T1 clustering closely with *T. nordicum* NR_184883.1 and receiving a bootstrap support value of 74%. Based on the BLAST similarity and phylogenetic placement, the aroma-producing isolate was identified as *Trichoderma nordicum*.

DISCUSSION

Soil represents an important reservoir of diverse filamentous fungi with considerable metabolic potential. In the present study, 20 fungal isolates were obtained from soil collected from Gautala Abhayaranya, and one isolate exhibiting a distinct characteristic aroma was selected for further molecular identification. The detection of an aroma-producing fungal isolate highlights the potential of soil-derived fungi as a source of volatile metabolites with possible biotechnological applications.

The selected isolate was identified through ITS sequence analysis. BLAST analysis showed the highest sequence similarity with *Trichoderma nordicum* (99.826% identity and 100% query coverage), while high similarity was also observed with other closely related *Trichoderma* species. Phylogenetic analysis further placed the isolate in close association with *T. nordicum*, supporting its identification. The close similarity observed among several *Trichoderma* taxa also demonstrates the limitations of ITS sequences for resolving closely related species within the genus. Therefore, although the present ITS-based analysis supports the identification of the isolate as *T. nordicum*, additional molecular markers such as translation elongation factor 1-alpha (TEF1-α) could provide stronger species-level resolution.

The characteristic aroma observed from the selected isolate is particularly significant because *Trichoderma* species are known to produce a diverse range of volatile organic compounds (VOCs). The production of VOCs in *Trichoderma* is influenced by factors including species or strain, culture conditions, growth stage, and interactions with other organisms.[3] Several *Trichoderma*-derived volatiles have been investigated for their biological activities, including antimicrobial and plant-growth-promoting effects. Among these, 6-pentyl-α-pyrone (6-PP), which has a characteristic coconut-like aroma, is one of the best-studied volatile compounds associated with *Trichoderma*. Recent literature indicates that 6-PP has been reported from multiple *Trichoderma* species and can constitute a substantial proportion of the VOC profile in some strains.[12]

However, the aroma detected in the present study should not be directly attributed to 6-PP or any other specific volatile compound because sensory evaluation alone cannot determine the chemical composition of fungal emissions. Recent studies demonstrate that *Trichoderma* isolates can produce complex mixtures of VOCs and that their profiles may vary considerably between isolates and culture conditions.[13] Therefore, the aroma observed in the present isolate provides a preliminary indication of volatile metabolite production rather than definitive evidence of a particular fragrance compound.

The isolation of an aroma-producing *T. nordicum* strain from soil is therefore of interest for further investigation. To the best of the scope of the present study, the observed aroma provides a basis for subsequent characterization of the volatile profile of the isolate. Future studies should employ headspace GC-MS or related analytical techniques to identify and quantify the VOCs responsible for the characteristic aroma. Optimization of culture conditions, including medium composition, incubation period, temperature, and other process parameters, could subsequently be investigated to enhance production of desirable volatile compounds. Such studies would help determine whether the isolate has potential as a microbial source of aroma or flavour-related compounds.

CONCLUSION

The present study successfully isolated 20 fungal isolates from soil, among which one isolate exhibited a distinct characteristic aroma. Based on ITS sequence analysis, BLAST similarity, and maximum-likelihood phylogenetic analysis, the selected isolate was identified as *Trichoderma nordicum*. The observation of characteristic aroma from the isolate indicates its potential for production of volatile metabolites of possible flavour and fragrance interest. However, the chemical nature of the aroma-producing compounds could not be established through sensory screening alone. Therefore, further studies involving headspace GC-MS analysis and optimization of culture conditions are required to identify and quantify the volatile compounds produced by the isolate and to assess its potential for flavour and fragrance applications.

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