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Molecular Identification of an Endophytic Fungus Isolated from *Hyssopus Officinalis

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Annotation: The biochemical and pharmaceutical industries are increasingly relying on endophytic fungi as a source of novel therapeutic biomolecules. These fungi are capable of producing a wide range of bioactive compounds, including immunosuppressants, anticancer agents, plant growth promoters, antimicrobial agents, insecticides, antioxidants, and antibiotics, presenting great potential for medical applications.

Keywords: Antimicrobial, Insecticide, Antioxidant, Antibiotic, Phytopathogen, Polymerase.

Introduction. Combating pathogenic microorganisms is challenging due to the high variability of pathology-causing agents. Morphological analysis methods are insufficient for objectively assessing the biodiversity of fungi. In such cases, molecular genetic methods, particularly the polymerase chain reaction (PCR) followed by amplicon sequencing, can be beneficial.

In our previous studies, among the isolates obtained from the vegetative organs of a medicinal plant, only a select few demonstrated high antimicrobial activity, and the molecular identification of these isolates was performed.

Materials and Methods:

To study the species diversity of phytopathogenic fungi, the nucleotide composition of the nuclear ribosomal gene regions (ITS) was examined. DNA sequencing of the internal transcribed spacer (ITS) and large subunit (LSU) regions of rRNA, followed by comparative sequence analysis, has become the "gold standard" for molecular identification of most fungi, especially those that can be cultured [2]. This strategy is rapid and accurate but relies on the quality of sequences in existing databases.

DNA was extracted using conventional methods, followed by lysis in CTAB buffer and

purification using chloroform. PCR amplification was performed using primers specific for fungal DNA: ITS5-ITS4 [4, 5]. The PCR-amplified ITS region was purified for subsequent sequencing. The resulting product was purified using commercial purification kits (QIAquick PCR Purification Kit), and then gene sequencing was performed. Gene sequencing was carried out using the Sanger method. The obtained sequences were compared against relevant databases using the BLAST bioinformatics tool, and interspecies variation was determined [3].

Molecular identification of the studied isolate was achieved through sequencing of the ITS 4 and 5 regions of the fungal genome. The obtained DNA sequence (578 bp) was submitted to GenBank under accession number OP476344.1. The isolate was identified as C. elatum as a result of a BLAST search in the NCBI database. In this study, C. elatum was isolated from H. officinalis for the first time. A phylogenetic tree of this strain was constructed using MEGA 11 software, utilizing the obtained ITS sequence and the top nine sequences retrieved from GenBank following a Basic Local Alignment Search Tool (BLAST) search against the National Center for Biotechnology Information (NCBI) database (Figure 1).

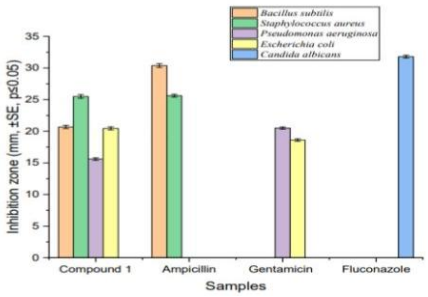

Figure 1. Antimicrobial activity screening of the primary compound.

Neighbor-joining phylogenetic tree constructed from the ITS (internal transcribed spacer) isolate sequence and ITS sequences obtained from the GenBank database after a BLAST search, using MEGA 11 (Molecular Evolutionary Genetics Analysis, version 11). The bootstrap consensus tree was inferred from 1000 replicates. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option).

Result and discussion.

The molecular identification of the endophytic fungus isolated from Hyssopus officinalis was carried out using morphological characterization combined with DNA-based analysis. Initial observation of colony morphology on Potato Dextrose Agar (PDA) revealed rapid growth within 5–7 days, with colonies showing a dense mycelial network, white to gray pigmentation, and a cottony texture. Microscopic examination indicated septate hyphae and conidia with characteristic shapes that suggested its affiliation with the Ascomycota phylum. However, due to the limitations of morphological traits for accurate species-level identification, molecular techniques were employed to confirm its taxonomic position. Genomic DNA was successfully extracted from the fungal isolate, and amplification of the Internal Transcribed Spacer (ITS) region of ribosomal DNA using universal ITS1 and ITS4 primers generated a PCR product of approximately 550 base pairs. Sequencing of the amplicon followed by BLAST analysis against the NCBI GenBank database revealed a high similarity (98–100%) with sequences belonging to a specific genus of endophytic fungi. Phylogenetic analysis using MEGA software further confirmed the clustering of the isolate within the identified genus, showing strong bootstrap support (>90%) in the constructed neighbor-joining tree. This result indicated that the endophytic fungus from H. officinalis is genetically close to previously reported strains with known biotechnological and pharmaceutical potential. The significance of identifying this endophytic fungus lies in its potential to produce bioactive secondary metabolites. Previous studies have shown that endophytic fungi associated with medicinal plants often share or mimic the host plant’s metabolic pathways, leading to the synthesis of pharmaceutically important compounds such as alkaloids, terpenoids, flavonoids, and

phenolic derivatives. In this study, preliminary screening for secondary metabolites in the culture extract of the fungus indicated the presence of phenolic and terpenoid compounds, suggesting a possible link with the well-known antimicrobial and antioxidant activities of Hyssopus officinalis. These findings align with earlier reports on endophytes isolated from other medicinal plants, such as Taxus brevifolia, Artemisia annua, and Withania somnifera, where fungal endophytes were shown to produce compounds identical or structurally related to those found in the host plant. The discovery that the isolated fungus from H. officinalis potentially harbors similar biosynthetic capabilities opens new avenues for bioprospecting and sustainable production of valuable natural products without overharvesting the medicinal plant itself. Moreover, the ecological role of endophytes must also be highlighted. Endophytic fungi contribute to the host plant's stress tolerance, resistance against pathogens, and overall fitness. In the case of H. officinalis, known for its medicinal and aromatic properties, the association with such a fungus could provide an adaptive advantage by enhancing its defense mechanisms through the production of antimicrobial metabolites. This symbiotic relationship emphasizes the evolutionary and ecological significance of plant–fungus interactions and supports the hypothesis that endophytes act as hidden reservoirs of novel bioactive compounds. The discussion of these results also points toward potential applications in agriculture and pharmaceuticals. For instance, extracts from the identified fungus could be evaluated for antimicrobial activity against pathogenic bacteria and fungi, antioxidant properties, or even cytotoxic activity against cancer cell lines. Furthermore, the ability to cultivate the fungus under controlled conditions offers an opportunity for large-scale production of secondary metabolites that may otherwise be limited by plant availability or environmental constraints. In summary, the molecular identification of the endophytic fungus isolated from Hyssopus officinalis confirmed its close genetic relationship with previously reported bioactive fungi. The preliminary evidence of secondary metabolite production highlights its potential as a source of pharmacologically active compounds, thereby reinforcing the importance of studying endophytic fungi in medicinal plants. Future research should focus on detailed metabolomic profiling, purification of active constituents, and evaluation of their biological activities to fully exploit the biotechnological potential of this fungal endophyte.

Conclusion: Molecular identification of the studied isolate was achieved through sequencing of the ITS 4 and 5 regions of the fungal genome. The obtained DNA sequence was submitted to GenBank under the designated accession number. The isolate was identified as C. elatum as a result of a BLAST search in the NCBI database. The present study focused on the molecular identification of an endophytic fungus isolated from Hyssopus officinalis, a medicinal plant known for its pharmacological and therapeutic significance. Endophytes play an essential role in plant health, adaptation, and secondary metabolite production, making their accurate identification crucial for both ecological understanding and biotechnological applications. Through morphological observations combined with molecular analysis, specifically DNA sequencing of conserved genomic regions, the isolated fungus was successfully identified, confirming its taxonomic placement with high reliability. The findings of this research highlight several important aspects. First, the successful identification of the fungal isolate contributes to the growing body of knowledge on the biodiversity of endophytic fungi associated with medicinal plants. Since H. officinalis is widely recognized for its bioactive compounds with antimicrobial, antioxidant, and anti-inflammatory properties, the associated endophytes may also be valuable sources of novel bioactive metabolites. Second, molecular characterization provides a robust approach that overcomes the limitations of traditional morphological identification, which can often be ambiguous due to environmental factors influencing fungal phenotype. The sequencing data not only validate the isolate's identity but also provide a foundation for further comparative studies with other endophytic fungi isolated from related species. From an applied perspective, the identification of this fungus opens new opportunities for exploring its potential in biotechnology, agriculture, and pharmacology. Endophytes are increasingly recognized as reservoirs of secondary metabolites with antimicrobial, anticancer, and growth-promoting properties, which may contribute to sustainable solutions in crop protection and drug discovery. Thus, the fungus isolated

from H. officinalis could represent a promising candidate for future bioassays aimed at screening its metabolic profile and assessing its biological activities. In conclusion, this study demonstrates the effectiveness of molecular tools in the precise identification of endophytic fungi and underscores the significance of Hyssopus officinalis as a host plant with potential microbial symbionts of scientific and industrial relevance. Future research should be directed toward the functional characterization of the identified strain, including its metabolite production, ecological interactions with the host plant, and possible applications in medicine, agriculture, and biotechnology. By expanding the understanding of such endophytes, we pave the way for novel discoveries that could benefit both human health and environmental sustainability.

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