



MOLECULAR IDENTIFICATION OF ENDOPHYTIC FUNGI ISOLATED FROM HYSSOPUS OFFICINALIS

Eshboev Farkhod

Senior Research Fellow, PhD,

Institute of the Chemistry of Plant Substances

Valieva Mufazzal

Master National University of Uzbekistan

Khayrullaeva Lobar

Assistant Lecturer,

Qarshi State University

Annotation: In recent years, endophytic fungi of medicinal plants have gained considerable attention as potential sources of novel bioactive compounds. These microorganisms, which reside asymptotically within plant tissues, are known to produce a wide range of secondary metabolites with antimicrobial, antifungal, and antioxidant properties. In this study, a highly active endophytic fungal isolate was obtained from the vegetative organs of *Hyssopus officinalis* and subjected to molecular identification. Using the ITS (Internal Transcribed Spacer) region sequencing and BLAST analysis against the NCBI GenBank database, the isolate was identified as *Colletotrichum elatum*. DNA extraction and sequencing were conducted using CTAB method, PCR amplification with ITS5-ITS4 primers, and Sanger sequencing. The obtained sequence was submitted to GenBank under the accession number OP476344.1. A phylogenetic tree was constructed using MEGA 11 software to confirm taxonomic affiliation. This study reports for the first time the isolation of *C. elatum* from *H. officinalis*, underlining its potential as a novel source of bioactive compounds.

Keywords: Endophytic fungi, *Hyssopus officinalis*, *Colletotrichum elatum*, secondary metabolites, ITS sequencing, phylogenetic analysis, antimicrobial activity, BLAST, molecular identification.

Introduction. The biochemical and pharmaceutical industries rely on endophytic fungi as a promising source of novel therapeutic biomolecules, which may include immunosuppressants, anticancer drugs, plant growth promoters, antimicrobial agents, insecticides, antioxidants, and antibiotics, thereby offering significant potential for medical applications [1]. Combating pathogenic microorganisms is complicated by the high variability of pathogenic agents. Morphological analysis methods are not sufficient for the objective assessment of fungal biodiversity. In such cases, molecular genetic approaches, particularly polymerase chain reaction (PCR) followed by sequencing of the amplicons, can be highly beneficial [3].

In our previous studies, among the isolates obtained from the vegetative parts of medicinal plants, only one demonstrated strong antimicrobial activity. Therefore, this isolate was subjected to molecular identification.

Results. To study the diversity of phytopathogenic fungi, the nucleotide composition of the nuclear ribosomal gene regions (ITS) was analyzed. Sequencing of the internal transcribed spacer (ITS) and the large subunit (LSU) regions of rRNA, followed by comparative sequence analysis, has become the "gold standard" for molecular identification of most fungi, particularly those that are cultivable [2]. This strategy is rapid and accurate but depends on the quality of the sequences available in existing databases.

DNA was extracted using conventional methods, lysed in CTAB buffer, and purified with chloroform. PCR amplification was performed using fungal-specific primers: ITS5–ITS4 [4,5]. The PCR-amplified ITS region was purified for subsequent sequencing. The amplified product was cleaned using a commercial purification kit (QIAquick PCR Purification Kit) and then subjected to gene sequencing. Sequencing was carried out using the Sanger method. The resulting sequences were compared against database entries using the BLAST bioinformatics tool to determine interspecies differences [3].

The molecular identification of the studied isolate was based on sequencing the ITS4 and ITS5 regions of the fungal genome. The obtained DNA sequence (578 bp) was submitted to GenBank under the accession number OP476344.1. A BLAST search in the NCBI database confirmed the isolate as *C. elatum*. This study reports *C. elatum* as being isolated from *H. officinalis* for the first time. A phylogenetic tree of this strain was constructed using MEGA 11 software, based on the obtained ITS sequence and the top nine related sequences from GenBank identified through a BLAST search in the National Center for Biotechnology Information (NCBI) database (Figure 1).

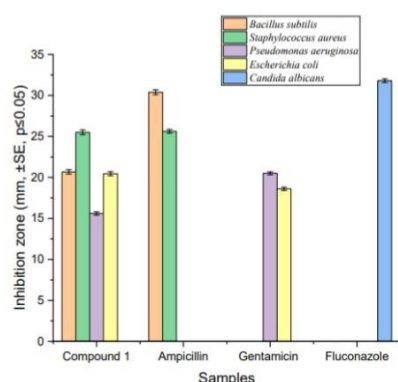


Figure 1. Antimicrobial Activity Assay of the First Compound

Phylogenetic tree constructed using the ITS (Internal Transcribed Spacer) sequence of the isolate obtained from the GenBank database after a BLAST search with MEGA 11 (Molecular Evolutionary Genetics Analysis, version 11). The neighbor-joining tree was generated based on 1000 bootstrap replicates. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option).

References:

1. Conrad L Schoch, Keith A Seifert, Sabine Huhndorf, Vincent Robert, John L Spouge, C André Levesque, Wen Chen; Fungal Barcoding Consortium; Fungal Barcoding Consortium Author List; Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi; 2012 April 7
2. White T.J, Bruns T, Lee S. & Taylor J. (1990) «Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics» page 13
3. Clement K.M. Tsui, James Woodhall, Wen Chen, K Andre Levesque, Anna Lau, Cor D Sean, Christiana Bashien, Mohammad Dj. Nadjafzadeh, G Sibren de Hoog. Molecular methods for the identification of pathogens and the detection of fungi in the environment. PMID: 22679603
4. Yurkov A.P., Kryukov A.A., Gorbunova A.O., Kojemyakov A.P., Stepanova G.V., Machs E.M., Rodionov A.V., Shishova M.F.. Molecular genetic identification of fungus arbuscular mycorrhiza. doi: 10.17816/ecogen16211-23\ Genetic basis of ecosystem evolution.
5. Kokaeva L.Yu., Elansky S.N., Beryozov Yu.I. Molecular identification of fungi - causing leaf spotting of potatoes and tomatoes in modern mycology in Russia. // «Sovremennaya mikologiya v Rossii» // Materialy 3-mejdunarodnogo mikologicheskogo forum. M., 2015. Volume 5, p. 66