DOI 10.66073/ReFocus-Conf/3010055

IDENTIFICATION AND PHYLOGENETIC PLACEMENT OF AN ENDOPHYTIC *ALTERNARIA* ISOLATE D15 FROM *FERULA KHUSTANICA*

Khamdamov Doniyor Shuxratovich[1] *and* **Eshboev Farhod Bakir o‘g‘li**[1]*
[1] *Institute for Advanced Studies at New Uzbekistan University, 100007 Tashkent, Uzbekistan*
*Corresponding author: f.eshboev@newuu.uz

Abstract: An endophytic fungal isolate, D15, recovered from *Ferula khustanica* (Apiaceae), was investigated to establish its taxonomic placement using internal transcribed spacer (ITS) sequencing. A 582-base reverse-strand Sanger read was compared with reference sequences from five Alternaria type strains, and a UPGMA phylogenetic tree was constructed. The isolate clustered within Alternaria and showed 99.2–99.8% sequence identity to the examined type strains. However, the low ITS divergence among these reference species prevents a reliable species-level assignment. The isolate is therefore designated Alternaria sp. D15 pending multilocus characterization.

Keywords: *Alternaria*; *Ferula khustanica*; endophytic fungi; ITS sequencing; molecular identification; phylogenetic analysis.

Introduction

Endophytic fungi colonize internal plant tissues and may represent valuable sources of structurally diverse secondary metabolites. Accurate taxonomic identification is essential before their biological and biosynthetic potential can be evaluated. The internal transcribed spacer (ITS) region is widely used as a fungal DNA barcode, although its resolution varies among taxonomic groups. This study aimed to determine the phylogenetic position of fungal isolate D15 obtained from Ferula khustanica and to assess whether the available ITS sequence supports identification at the species level.

Materials and Methods

The ITS region of isolate D15 was analyzed using a 582-base reverse-strand Sanger sequencing read spanning the ITS4–ITS5 region. The sequence was compared by semi-global alignment with ITS sequences of five *Alternaria* type strains retrieved from the NCBI RefSeq targeted-loci database: *A. destruens* (NR_137143.1), *A. alstroemeriae* (NR_163686.1), *A. doliconidium* (NR_158361.1), *A. tectorum* (NR_187081.1), and *A. eichhorniae* (NR_111832.1). *Pseudophaeosphaeria rubi* (NR_154351.1) was used as an outgroup. A UPGMA tree was reconstructed using pairwise p-distances.

Results

Phylogenetic analysis placed isolate D15 within the genus *Alternaria* (Figure 1). Its ITS sequence differed from *A. destruens* at 1 of 521 aligned positions (99.8% identity), from *A. alstroemeriae* and *A. doliconidium* at 2 of 530 and 2 of 529 positions, respectively (99.6%), from *A. tectorum* at 3 of 520 positions (99.4%), and from *A. eichhorniae* at 4 of 521 positions (99.2%). The outgroup exhibited 17.6% sequence divergence. The five *Alternaria* type strains differed from one another by only 0.2–0.8% in the analyzed region.

FOR CITATION
Khamdamov D., Eshboev F. Identification and phylogenetic placement of an endophytic Alternaria isolate D15 from Ferula khustanica // Proceedings of the Republican Scientific and Practical Conference “Status and Development Prospects of Fundamental and Applied Microbiology: The Viewpoint of Young Scientists”. – Tashkent, 2026. – P. 203–204.
DOI: https://doi.org/10.66073/ReFocus-Conf/3010055

Discussion

The close similarity of the examined ITS sequences supports assignment of D15 to *Alternaria* but does not allow confident discrimination among the reference species. Although *A. destruens* showed the highest sequence identity, this observation alone is insufficient to identify D15 as that species. Additional sequence data from more informative loci, such as GAPDH, RPB2, and TEF1, are needed for a robust species-level determination.

Conclusion

ITS sequencing and phylogenetic analysis identified D15 as a member of the genus *Alternaria*. Given the limited discriminatory power of the available ITS region, the isolate should be reported as *Alternaria* sp. D15 until multilocus analysis provides a more precise taxonomic assignment.

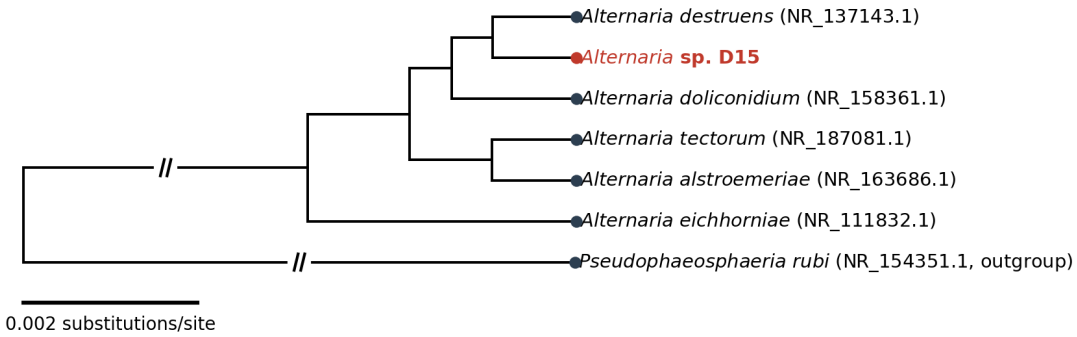


Figure 1. UPGMA tree based on ITS sequences showing the placement of *Alternaria sp*. D15 (red) relative to five *Alternaria* type strains, with *Pseudophaeosphaeria rubi* as the outgroup. Basal branches are truncated (//)

References

1. Dettman J.R., Eggertson Q.A., Kim N.E. Species diversity and molecular characterization of Alternaria section Alternaria isolates collected mainly from cereal crops in Canada // Frontiers in Microbiology. – 2023. – Vol. 14. – Art. 1194911. – DOI: 10.3389/fmicb.2023.1194911.
2. Parvandi M., Rezadoost H., Farzaneh M. Introducing Alternaria tenuissima SBUp1, as an endophytic fungus of Ferula assa-foetida from Iran, which is a rich source of rosmarinic acid // Letters in Applied Microbiology. – 2021. – Vol. 73, No. 5. – P. 569–578. – DOI: 10.1111/lam.13542.
3. Raja H.A., Miller A.N., Pearce C.J. et al. Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community // Journal of Natural Products. – 2017. – Vol. 80, No. 3. – P. 756–770. – DOI: 10.1021/acs.jnatprod.6b01085.
4. Safaie N., Salehi M., Felegari M. et al. Culture-based diversity of endophytic fungi of three species of Ferula grown in Iran // Frontiers in Microbiology. – 2024. – Vol. 15. – Art. 1363158. – DOI: 10.3389/fmicb.2024.1363158.