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ITS-BASED IDENTIFICATION AND PHYLOGENETIC CHARACTERIZATION OF ENDOPHYTIC *ALTERNARIA SP.* H8 ISOLATED FROM *HELICHRYSUM MUSSAE*

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Abstract: An endophytic fungal isolate, H8, recovered from *Helichrysum mussae*, was investigated using internal transcribed spacer (ITS) sequence analysis to clarify its taxonomic placement. A quality-trimmed, 544-bp reverse-strand ITS read was compared with reference sequences of five *Alternaria* type strains. Phylogenetic analysis placed H8 within the genus *Alternaria*. The isolate matched the *A. destruens* type strain across 521 aligned positions and shared 99.4–99.8% sequence identity with four additional species. Because the ITS region showed limited variation among the reference taxa, these findings support identification at the genus level but do not establish a definitive species assignment. Additional multilocus sequencing is required for species-level resolution.

Keywords: endophytic fungi; *Helichrysum mussae*; *Alternaria*; ITS sequencing; fungal identification; phylogenetic analysis.

Introduction

Endophytic fungi colonize internal tissues of apparently healthy plants and represent a valuable resource for exploring fungal diversity and specialized metabolism. Accurate taxonomic characterization is essential before assessing their biological activities or biotechnological applications. Although the internal transcribed spacer (ITS) region is widely used as a fungal DNA barcode, closely related species may share highly similar ITS sequences. This study aimed to determine the phylogenetic position of fungal isolate H8 obtained from *Helichrysum mussae* and to evaluate the taxonomic resolution provided by its ITS sequence.

Materials and Methods

A single reverse-strand ITS sequence of 544 bp was obtained from isolate H8 after quality trimming (mean Phred score: 34). The sequence was aligned with ITS reference sequences from five *Alternaria* type strains: *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) served as the outgroup. Four ambiguous nucleotide calls were treated as compatible with their corresponding aligned reference bases. Sequence similarity and phylogenetic placement were evaluated using the aligned ITS region.

Results

The ITS-based phylogenetic analysis positioned isolate H8 within *Alternaria* (Figure 1). The sequence was identical to that of the *A. destruens* type strain across 521 aligned positions. Comparisons with *A. alstroemeriae* and *A. doliconidium* each revealed one difference (99.8%

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identity), whereas *A. tectorum* and *A. eichhorniae* differed at two (99.6%) and three (99.4%) positions, respectively. The outgroup showed 17.9% sequence divergence.

Discussion

The close ITS similarity between H8 and multiple *Alternaria* type strains indicates that this marker alone cannot reliably discriminate among the examined species. Differences relative to *A. alstroemeriae*, *A. doliconidium*, and *A. tectorum* involved single-base length variants in adenine-rich regions, while *A. eichhorniae* showed two additional substitutions. Consequently, the proximity of H8 to *A. destruens* in the phylogenetic tree should not be interpreted as conclusive evidence of species identity. Analysis of additional loci, including GAPDH, RPB2, and TEF1, would provide greater taxonomic resolution.

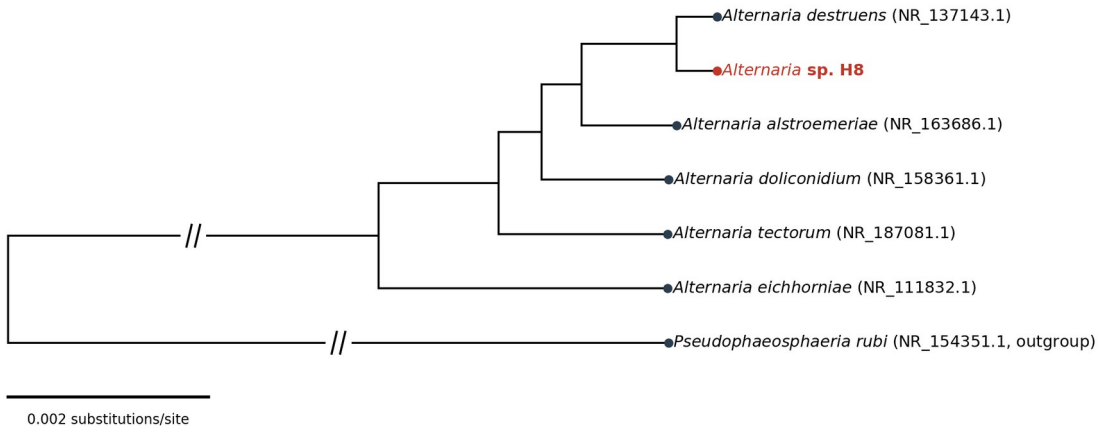


Figure 1. ITS-based phylogenetic tree showing the placement of *Alternaria sp.* H8 (red) among *Alternaria* type strains, with *Pseudophaeosphaeria rubi* as the outgroup. Truncated basal branches are indicated by //. Scale bar: 0.002 substitutions per site

Conclusion

ITS sequence comparisons support assignment of the endophytic isolate H8 to the genus *Alternaria*. However, the limited sequence divergence among closely related reference species prevents confident species-level identification. The isolate should therefore be designated *Alternaria sp.* H8 pending multilocus confirmation.

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