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IDENTIFICATION AND PHYLOGENETIC PLACEMENT OF THE FUNGAL ISOLATE S14 FROM *SILYBUM MARIANUM* WITHIN *ASPERGILLUS* SECTION *NIGRI*

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Abstract: Endophytic fungal isolate S14 from *Silybum marianum* was examined using a 340-bp ITS sequence and compared with reference sequences of *Aspergillus* section Nigri type strains. Phylogenetic analysis placed S14 within section *Nigri*, with 98.5% sequence identity to five closely related type strains. Because the analyzed ITS region did not discriminate among these species, the isolate was designated *Aspergillus sp*. (section Nigri) S14 pending confirmatory sequencing and analysis of additional loci.

Keywords: *Silybum marianum*; endophytic fungi; *Aspergillus* section Nigri; ITS sequencing; phylogenetic analysis; fungal identification.

Introduction

Endophytic fungi inhabit healthy plant tissues without causing visible symptoms and are an established source of structurally diverse secondary metabolites. Medicinal plants such as *Silybum marianum* (milk thistle), known for silymarin, are attractive hosts for bioprospecting. Reliable isolate identification is a prerequisite for such investigations. The internal transcribed spacer (ITS) region is the standard first-line fungal DNA barcode. This study aimed to identify isolate S14 recovered from *S. marianum* using ITS sequence analysis and to assess whether the available barcode supports species-level identification.

Materials and Methods

A single ITS4-primed sequence read of 340 bp, spanning the 5.8S rRNA gene and adjacent ITS regions, was obtained from isolate S14. The sequence was compared with six *Aspergillus* section *Nigri* type-strain sequences from the NCBI RefSeq targeted-loci database: *A. niger* (NR_111348.1), *A. tubingensis* (NR_131293.1), *A. luchuensis* (NR_135449.1), *A. costaricensis* (NR_103604.1), *A. foetidus* (NR_163668.1), and *A. hubkae* (NR_200571.1). *Aspergillus flavus* (NR_111041.1; section Flavi) served as an outgroup. Phylogenetic relationships were visualized using a UPGMA tree.

Results

Isolate S14 clustered within *Aspergillus* section *Nigri* (Figure 1). It differed from the type strains of *A. niger*, *A. tubingensis*, *A. luchuensis*, *A. costaricensis*, and *A. foetidus* at five of 341 alignment positions (98.5% identity), and from *A. hubkae* at ten of 343 positions (97.1% identity). Divergence from the *A. flavus* outgroup was 7.5%. The five closest reference sequences were identical throughout the analyzed window, whereas *A. hubkae* differed from them at five positions.

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Discussion

The short ITS window cannot distinguish the five closest species. All five observed differences between S14 and its nearest references occurred in G/C-rich tracts, consisting of three single-nucleotide insertions or deletions and one adjacent GC/CG exchange. These differences may arise from base-calling uncertainty in a single sequence read and should not be interpreted as species-specific markers without sequencing the opposite strand. Further analysis of calmodulin and β-tubulin loci is needed to resolve the isolate at species level.

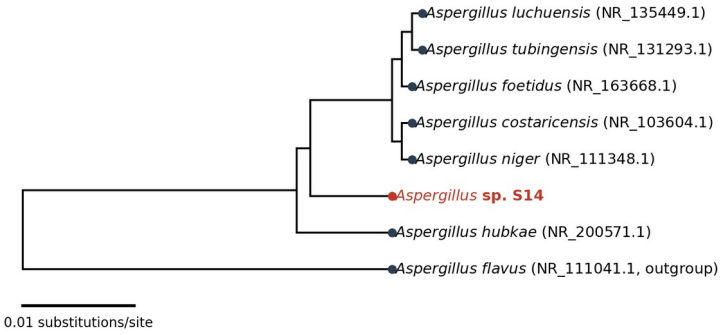


Figure 1. UPGMA tree of the ITS region (340-bp window) placing *Aspergillus sp*. S14 (red) among *Aspergillus* section *Nigri* type strains; *A. flavus* (section Flavi) is the outgroup. Scale bar: 0.01 substitutions per site

Conclusion

The available ITS sequence supports assignment of isolate S14 to *Aspergillus* section *Nigri* but does not permit reliable species-level identification. Until complementary sequence data and additional genetic markers become available, the isolate should be designated *Aspergillus sp*. (section Nigri) S14.

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