

Identification of a Fungal 1,8-Cineole Synthase from *Hypoxylon* sp. with Specificity Determinants in Common with the Plant Synthases*

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Capsule

Background: 1,8-Cineole, a commercially important monoterpene, was identified as a fungal product.

Results: The 1,8-cineole synthase was identified from a *Hypoxylon* fungal genome, and mutagenesis revealed a critical asparagine residue.

Conclusion: The fungal 1,8-cineole synthase uses a mechanism similar to the plant version.

Significance: This is the first identified fungal monoterpene synthase and may facilitate future terpene synthase identification and production.

Abstract

Terpenes are an important and diverse class of secondary metabolites widely produced by fungi. Volatile compound screening of a fungal endophyte collection revealed a number of isolates in the family Xylariaceae, producing a series of terpene molecules, including 1,8-cineole. This compound is a commercially important component of eucalyptus oil used in pharmaceutical applications and has been explored as a potential biofuel additive. The genes that produce terpene molecules, such as 1,8-cineole, have been little explored in fungi, providing an opportunity to explore the biosynthetic origin of these compounds. Through genome sequencing of cineole-producing isolate E7406B, we were able to identify 11 new terpene synthase genes. Expressing a subset of these genes in *Escherichia coli* allowed identification of the *hyp3* gene, responsible for 1,8-cineole biosynthesis, the first monoterpene synthase discovered in fungi. In a striking example of convergent evolution, mutational analysis of this terpene synthase revealed an active site asparagine critical for water capture and specificity during cineole synthesis, the same mechanism used in an unrelated plant homologue. These studies have provided insight into the evolutionary relationship of fungal terpene synthases to those in plants and bacteria and further established fungi as a relatively untapped source of this important and diverse class of compounds.

Enzyme Mechanism Fungi Mutagenesis Phylogenetics Terpenoid

Footnotes

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) JYCQ00000000.

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