

A fivefold parallelized biosynthetic process secures chlorination of *Armillaria mellea* (honey mushroom) toxins.

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Abstract

The basidiomycetous tree pathogen *Armillaria mellea* (honey mushroom) produces a large variety of structurally related antibiotically active and phytotoxic natural products, referred to as the melleolides. During their biosynthesis, some members of the melleolide family of compounds undergo monochlorination of the aromatic moiety, whose biochemical and genetic basis was not known previously. This first study on basidiomycete halogenases presents the biochemical in vitro characterization of five flavin-dependent *A. mellea* enzymes (ArmH1 to ArmH5) that were heterologously produced in *Escherichia coli*. We demonstrate that all five enzymes transfer a single chlorine atom to the melleolide backbone. A 5-fold, secured biosynthetic step during natural product assembly is unprecedented. Typically, flavin-dependent halogenases are categorized into enzymes acting on free compounds as opposed to those requiring a carrier-protein-bound acceptor substrate. The enzymes characterized in this study clearly turned over free substrates. Phylogenetic clades of halogenases suggest that all fungal enzymes share an ancestor and reflect

a clear divergence between ascomycetes and basidiomycetes.

Involved units

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