

Biosynthesis of the Halogenated Mycotoxin Aspirochlorine in Koji Mold Involves a Cryptic Amino Acid Conversion.

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Abstract

Aspirochlorine (1) is an epidithiodiketopiperazine (ETP) toxin produced from koji mold (*Aspergillus oryzae*), which has been used in the oriental cuisine for over two millennia. Considering its potential risk for food safety, we have elucidated the molecular basis of aspirochlorine biosynthesis. By a combination of genetic and chemical analyses we found the *acl* gene locus and identified the key role of AclH as a chlorinase. Stable isotope labeling, biotransformation, and mutational experiments, analysis of intermediates and an in vitro adenylation domain assay gave totally unexpected insights into the *acl* pathway: Instead of one Phe and one Gly, two Phe units are assembled by an iterative non-ribosomal peptide synthetase (NRPS, AclP), followed by halogenation and an unprecedented Phe to Gly amino acid conversion. Biological assays showed that both amino acid transformations are required to confer cytotoxicity and antifungal activity to the mycotoxin.

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