BIOTRANS
LA ROCHELLE 2023

N°1516 / PC
TOPIC(s) : Enzyme discovery and engineering

Discovery of the biosynthetic enzymes for the fatty acid-rearranged natural products fischerazoles A-C from a cyanobacterium

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PURPOSE OF THE ABSTRACT

Cyanobacteria are prolific producers of secondary metabolites and hold a large amount of unexplored biosynthetic gene clusters (BGCs) encoding enzymes with potential for biotechnological or pharmaceutical application. Using our recently developed metabolite discovery approach that is based on stable isotope-labeled fatty acids and comparative metabolomics, we uncovered novel compounds from the cyanobacterium Fischerella sp. PCC 9431. These new lipopeptides, fischerazoles A-C, had particularly interesting structural features such as extensive chlorination of the fatty acyl chain, a rare gem-dichlorovinylidene moiety and an unusual terminal methylated carboxamide. Most strikingly, they contained a pendant allyl alcohol which, according to our supplementation experiments with stable isotope-labeled precursors, derived from fatty acid rearrangement during the biosynthesis. By mining the genome of PCC 9431 for halogenases, we detected the corresponding BGC (fsh) with three halogenase homologs and a polyketide synthase (PKS)/ non-ribosomal peptide synthetase (NRPS) core. Additional biosynthetic enzymes included a fatty acyl-AMP ligase (FAAL) for initiation of the biosynthesis by fatty acid activation and loading to a carrier protein, a fatty acid desaturase, a SAM-methyltransferase and a cytochrome P450, likely involved in the functionalization of a mid-chain aliphatic carbon to create the pendant allyl alcohol moiety. Although the SAM methyltransferase did not cluster with any characterized enzymes in sequence similarity networks, bioinformatic analysis and in vitro experiments suggest that it creates a cyclopropane on a thioester-tethered palmitoleic acid substrate. We hypothesize that this cyclopropanyl fatty acid is then used as a substrate by the cytochrome P450 to perform a hydroxylation and rearrangement reaction. By uncovering structurally novel natural products, we here discovered several cyanobacterial biosynthetic enzymes that could ultimately expand the biocatalyst's toolbox.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

natural products | cyanobacteria | methyltransferase | P450

BIBLIOGRAPHY