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New insights into cichorine biosynthesis in Aspergillus nidulans through bioinformatic and metabolomic analyses

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Cichorine is an isoindolinone alkaloid mainly produced by fungi such as *Aspergillus nidulans*. Its derivatives have been reported to display diverse biological activities, including antioxidant, antimicrobial, cytotoxic, and antiproliferative effects against several cancer cell lines[1]. Cichorine originates from 3-methylorsellinic acid (3-MOA), and although its biosynthetic gene cluster (BGC) has been described in *A. nidulans*[2], the functional roles of several genes remain only partially understood, particularly those involved in nitrogen incorporation and oxidation steps. In this work, it was combined bioinformatic with metabolomic analyses of *A. nidulans* mutant strains to further characterize the cichorine biosynthetic pathway. The *A. nidulans* genome (NCBI accession: GCA_000011425.1) was screened with antiSMASH, and Cic BGC protein sequences were analyzed by BLASTp and InterPro. In earlier studies, the Cic BGC was overexpressed, yielding strain NID792, which served as the background for constructing targeted gene deletions. Mutant strains included NID952 (ΔAN6444), NID954 (ΔAN6449), NID956 (ΔAN6447), NID1012 (ΔAN6448), and NID1010 (ΔAN6445). Extracts from these strains were subjected to UHPLC-ESI-HRMS/MS analyses in both positive and negative ionization modes. AntiSMASH predicted 18 genes in the Cic BGC. However, comparative sequence analysis indicated that only five genes are directly involved in cichorine biosynthesis: AN6448 (polyketide synthase), AN6447 (*O*-methyltransferase), AN6445 (GMC oxidoreductase, 49% identity to PatE), AN6449 (CYP450 monooxygenase), and AN6444 (nonribosomal peptide synthase-like – NRPS-like, 40% identity to StbB). The function of AN6448, AN6447, AN6445, and AN6449 had been previously confirmed through metabolomic analyses of mutants[2] and corroborates with bioinformatic and metabolomic results obtained here. Additionally, it was proposed that the uncharacterized AN6444 Cic gene is involved in the conversion of 3-MOA to its corresponding aldehyde in early steps of cichorine biosynthesis.

Keywords: *Cichorine biosynthesis, CRISPR/Cas, HPLC-MS/MS metabolomics.*

References: *[1]Bailly. Eur. J. Med. Chem. Rep., 9, 100112, 2023.; [2]Sanchez et al. Med. Chem. Comm., 3, 997, 2012.*

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