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ASPERGILLUS TUBINGENSIS S23 AS A PROMISING SOURCE OF PHARMACEUTICALLY IMPORTANT SECONDARY METABOLITES

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Abstract: The biosynthetic potential of *Aspergillus tubingensis* S23, an endophytic fungus isolated from the medicinal plant *Eremostachys isochila*, was evaluated through whole-genome sequencing and comparative genome mining. Molecular phylogeny and phylogenomics supported its identification as *A. tubingensis*. antiSMASH predicted 95 biosynthetic gene cluster (BGC) regions, including regions similar to characterized clusters associated with kojic acid, yanuthone D, clavaric acid, TAN-1612, nidulanin A, and burnettiene A. These results indicate substantial potential for genome-guided natural-product discovery, although the production and biological activities of the predicted compounds require experimental verification.

Keywords: *Aspergillus tubingensis*; endophytic fungi; secondary metabolites; biosynthetic gene clusters; kojic acid; natural-product discovery.

Introduction

Endophytic fungi represent a promising source of structurally diverse secondary metabolites with potential applications in pharmaceutical biotechnology and drug discovery. These microorganisms inhabit internal plant tissues without causing apparent disease symptoms and possess complex biosynthetic pathways responsible for producing numerous biologically active compounds. Among endophytic fungi, species belonging to the genus *Aspergillus* are particularly attractive because of their ability to synthesize polyketides, nonribosomal peptides, terpenoids, and other specialized metabolites exhibiting a wide range of biological activities. However, the pharmaceutical potential of many fungal isolates remains insufficiently explored, particularly at the genomic and biosynthetic levels. The integration of whole-genome sequencing, biosynthetic gene cluster (BGC) mining, and metabolite characterization offers a powerful approach for identifying promising microbial producers of pharmaceutically relevant natural products. The present study investigates the biosynthetic potential of *Aspergillus tubingensis* S23, an endophytic fungus isolated from the medicinal plant *Eremostachys isochila*, with particular emphasis on its capacity to produce secondary metabolites of potential pharmaceutical importance.

Materials and Methods

The fungal strain S23 was isolated from healthy, asymptomatic tissues of *Eremostachys isochila* (Lamiaceae), a medicinal plant collected in Uzbekistan. Taxonomic identification was performed using internal transcribed spacer (ITS) sequencing, multilocus phylogenetic analysis of three protein-coding genes (*benA*, *CaM*, and *RPB2*), and genome-scale phylogenomic analysis. Whole-genome sequencing was conducted using the MGI DNBSEQ-T7 platform with 150 bp paired-end reads. Sequencing data were quality-filtered using fastp, and genome assembly was performed

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using SPAdes v3.15.5. The biosynthetic potential of the strain was evaluated using antiSMASH v8.0.4, while predicted gene clusters were compared with experimentally characterized biosynthetic pathways available in the MIBiG database.

Results and Discussion

Molecular identification and phylogenomic analyses confirmed that strain S23 belongs to *Aspergillus tubingensis*, a member of the section *Nigri*. Genome mining revealed substantial biosynthetic diversity, with 95 predicted biosynthetic gene cluster regions, including clusters associated with polyketide synthases, nonribosomal peptide synthetases, terpene biosynthesis, and other specialized metabolic pathways. This extensive biosynthetic repertoire indicates the potential of *A. tubingensis* S23 to produce chemically diverse secondary metabolites, including compounds of pharmaceutical interest.

Comparative analysis of the predicted BGCs revealed several pathways associated with known bioactive fungal metabolites, including yanuthone D, burnettiene A, nidulanin A, TAN-1612, and kojic acid. Notably, the yanuthone D-associated BGC was found to be complete in strain S23, whereas corresponding genomic regions were disrupted in its closest relatives (Table 1).

This observation suggests distinctive biosynthetic capabilities that may differentiate S23 from other closely related strains. The burnettiene A-associated cluster exhibited a distribution consistent with vertical inheritance and differential gene loss (Figure 1), while clusters related to nidulanin A and TAN-1612 were broadly conserved across the examined fungal lineages. In contrast, the kojic acid biosynthetic pathway showed lineage-specific patterns of gene retention, relocation, and loss, highlighting the evolutionary plasticity of fungal secondary metabolism.

Table 1. Biosynthetic gene clusters in strain S23 with similarity to characterized compounds of reported pharmaceutical, cosmetic, or antimicrobial potential

Compound	MIBiG similarity	Chemical class	Documented/reported bioactivity
Kojic acid	66%	Pyranone	Cosmetic potential, Antioxidant, Antimicrobial
Yanuthone D	90%	Meroterpenoid	Antifungal activity
Clavaric acid	100%	Triterpenoid	Anticancer activity
TAN-1612	100%	Anthraquinone-type polyketide	Broadly reported for antimicrobial and anticancer bioactivity
Nidulanin A	75%	Polyketide–nonribosomal peptide hybrid	Secondary metabolite of Aspergillus nidulans, bioactivity potential is uncharacterized.
Burnettiene A	100%	Polyketide	Bioactive potential has not yet been reported.

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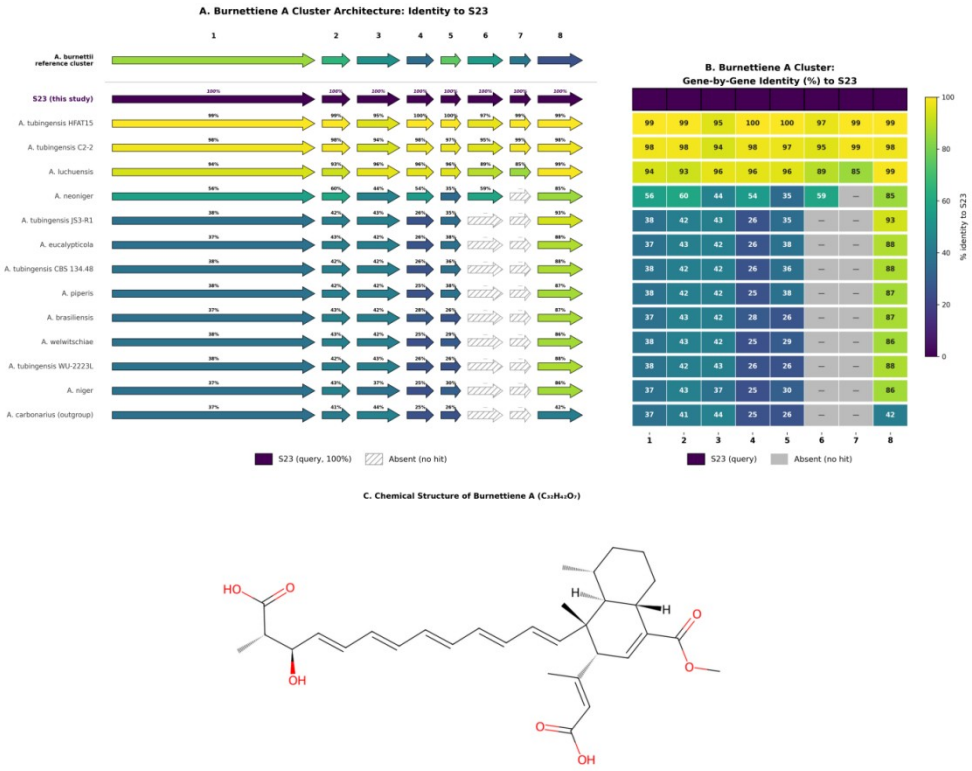


Figure 1. Burnettiene A biosynthetic gene cluster comparative and evolutionary analysis. (A) Gene architecture across all 14 genomes compared to the *A. burnettii* reference cluster (BGC0002139)

The findings demonstrate that *Aspergillus tubingensis* S23 possesses considerable potential as a microbial source of pharmaceutically relevant secondary metabolites. The identification of 95 predicted biosynthetic gene clusters, including pathways associated with kojic acid, yanuthone D, burnettiene A, nidulanin A, and TAN-1612, highlights the strain's extensive biosynthetic capacity. These results establish *A. tubingensis* S23 as a promising candidate for genome-guided natural-product discovery and provide a foundation for future metabolomic investigations, isolation of novel bioactive compounds, and development of potential pharmaceutical applications.

Discussion

The diverse predicted BGC repertoire suggests that S23 may synthesize multiple classes of specialized metabolites. The conservation and divergence of individual clusters across related genomes highlight the evolutionary complexity of fungal secondary metabolism. However, sequence similarity to characterized BGCs does not establish the production, chemical identity, yield, or pharmacological activity of the corresponding metabolites in S23. Metabolomic profiling, compound isolation, and targeted bioactivity assays are required to validate these predictions.

Conclusion

Aspergillus tubingensis S23 harbors 95 predicted BGC regions and several candidate pathways related to compounds of pharmaceutical or biotechnological interest. The findings support its selection for further genome-guided metabolite discovery and experimental characterization.

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