

Advancing Endophyte Studies through Multi-Omics Excellence

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SUMMARY

Omics techniques, comprising genomics, transcriptomics, proteomics, and metabolomics, play a pivotal role in the identification and characterization of novel bioactive compounds produced by fungal endophytes, along with their potential applications. These advanced methodologies contribute substantially to unravelling the intricate interactions between fungal endophytes and their host plants. Consequently, they provide valuable insights into the molecular mechanisms underpinning these relationships and shed light on the potential applications of the derived bioactive compounds. This integrative scientific approach is instrumental in harnessing the full potential of fungal endophytes for various beneficial applications across diverse fields.

INTRODUCTION

Fungal endophytes represent valuable reservoirs of bioactive compounds with diverse applications. The exploration of these compounds not only enhances our comprehension of ecological interactions but also holds significant potential for the development of novel products with agricultural, medicinal, and industrial relevance. Transcriptomics, proteomics, and metabolic technologies have emerged as valuable tools for elucidating plant metabolic pathways and unravelling the regulatory mechanisms of crucial genes governing these processes (Biradar *et al.*, 2023). Continuous investigation into the diversity of fungal endophytes and an in-depth understanding of the ecological roles played by bioactive compounds present opportunities for groundbreaking discoveries and practical applications.

Genomics

High-throughput sequencing technologies, exemplified by next-generation sequencing (NGS) platforms like Illumina, PacBio, and Oxford Nanopore, have played a pivotal role in unveiling the genomic landscape of endophytic fungi. Specifically, whole-genome sequencing of fungal endophytes facilitates a comprehensive understanding of their genetic constitution, potential for secondary metabolite production, and adaptations to the host environment. Comparative genomic analyses across different endophytes contribute to the identification of conserved genes, unique features, and potential virulence or symbiotic factors. *Calcarisporium arbuscula*, a fungal endophyte residing in the fruit bodies of Russulaceae, has garnered attention for its production of various secondary metabolites with antibiotic, anti-carcinogenic, and anti-nematode activities. Notably, it synthesizes a significant quantity of aurovertin-type mycotoxins, particularly aurovertin B, recognized as a potent therapeutic anti-carcinogenic agent, and aurovertin D, which exhibits strong efficacy against root knot nematodes (*Meloidogyne incognita*). These attributes position *Calcarisporium arbuscula* as a promising candidate for biocontrol and the development of therapeutic agents in medical contexts. (Varma *et al.*, 2024)

Sixty-five gene clusters associated with the synthesis of secondary metabolites in the endophytic fungal strain *Calcarisporium arbuscula* NRRL 3705 were identified using antiSMASH 4.0. The researchers also predicted various gene clusters involved in mycotoxin production, including alternariol, citrinin, aflatoxin, isoflavipucine, and destruxin. Among these clusters, twenty-three contained genes for polyketide synthases (PKS), while the remaining twelve harbored genes encoding non-ribosomal peptide synthases (NRPS). Gene expression analysis, based on RNA sequencing and FPKM metrics, revealed that the PKS gene exhibited high expression levels compared to other genes in the identified clusters. This suggests that a majority of gene clusters are either silent or expressed at low levels. (Cheng *et al.*, 2021)

The omics-based data, coupled with bioinformatics analyses, unveiled the extensive repertoire of biosynthetic gene clusters in *Calcarisporium arbuscula* NRRL 3705, with the potential to produce a diverse array of secondary metabolites. The genome, sequenced using high-quality Single Molecule Real-Time (SMRT) sequencing technology, was assembled with an approximate size of 45.01 Mb. This genomic information holds promise for comparative genomics analyses and the discovery of novel genes in fungal endophytes, paving the way for future trends in secondary metabolite exploration using omics tools. (Varma *et al.*, 2024)

Transcriptomics

Transcriptomics, a subset of molecular biology, focuses on scrutinizing an organism's transcriptome, encompassing all RNA molecules produced by its genome at any given time. This includes messenger RNA (mRNA), non-coding RNA (ncRNA), and small RNA. RNA-Seq, a widely employed technique in transcriptomics, is utilized to comprehensively analyze the transcriptome of fungal endophytes. It furnishes details on gene expression levels, alternative splicing, and novel transcripts across different conditions, offering insights into dynamic changes in gene expression during interactions with the host.

Microarray technology is another tool for transcriptomic analysis, enabling simultaneous measurement of expression levels for thousands of genes. Transcriptomics aids in pinpointing genes upregulated or downregulated under specific conditions or stimuli. Differential gene expression analysis is particularly crucial for understanding alterations in gene expression patterns across various biological states.

An early transcriptome-focused study delved into genes associated with pyrimidine metabolism in *Epulorhiza* sp., a fungal endophyte isolated from *Anoectochilus roxburghii* orchid roots. Another investigation highlighted significantly differentially expressed antifungal genes in the endophytic fungus *Epichloe festucae* integrated with the grass *Festuca rubra*, shedding light on molecular interactions and gene expression changes in the endophyte-grass symbiotic relationship.

Integrated omics approaches, combining genomics, transcriptomics, and metabolomics, were employed to analyze genes linked to cellulose biodegradation and biofuel production in the fungal endophyte *Ascocoryne sarcooides*. Dual RNA-sequencing data uncovered that co-culturing bacterial and fungal strains regulates metabolic and developmental processes in both organisms. This regulation may involve adjustments in organic sulfur equilibrium through methionine acquisition. (Varma *et al.*, 2024)

Proteomics

Proteomics, the comprehensive investigation of the complete repertoire of proteins synthesized by an organism, tissue, or cell at a specific temporal point, is instrumental in identifying and characterizing the proteins generated by fungal endophytes. This field encompasses the identification, characterization, and quantification of proteins to elucidate their functions and interactions. The integration of 2D-gel electrophoresis and mass spectrometry stands as a widely embraced approach in proteomic analyses. Presently, Mass Spectrometry-based proteomics emerges as a powerful technique for mapping the entirety of proteins produced by fungal endophytes. This method provides profound insights into their interactions with host plants, unravelling biological pathways and post-translational modifications.

A notable application of Mass Spectrometry-based proteomics involves the examination of the complete protein set in *Atractylodes lancea*, both inoculated with and without the presence of the endophytic fungal strain *Gilmaniella* sp. AL12. The investigation reveals the upregulation of proteins associated with carbon fixation, carbohydrate metabolism, and energy metabolism. This suggests that the endophyte *Gilmaniella* sp. AL12 may play a pivotal role in promoting biomass in *Atractylodes lancea*, shedding light on the potential impacts of endophytic fungi on host plant physiology. (Varma *et al.*, 2024)

Metagenomics

Metagenomics plays a pivotal role in advancing the understanding of fungal endophytes by providing a robust tool for investigating their diversity, functional capabilities, and ecological roles within plant hosts and associated environments. This approach facilitates the comprehensive exploration of fungal diversity in plant tissues and other environmental samples, allowing for the identification of distinct fungal species and their relative abundance. Metagenomics, particularly when coupled with high-throughput technologies, enables in-depth analyses of microbial structure, function, and community dynamics.

These technologies find practical application in screening fungal endophytic strains. Traditional approaches for analyzing fungal communities were initially culture-based, supplemented by ITS region sequencing. However, these methods were limited in their ability to process a sufficient number of samples. Presently, metagenomics, complemented by computational tools, stands as a robust molecular approach for identifying functional genes in fungal endophytes associated with host plants.

Ephedra sinica serves as a model plant for investigating the intricate relationship between fungal endophytes and various tissues, each exhibiting distinct biological potencies. A systematic metagenomic and metabolomic study was conducted to elucidate the community of fungal endophytes and their associated metabolic compounds in the roots and stem of *Ephedra sinica*. The findings unveiled that fungal strains OTU30589 and OTU48 were

identified as endophytes in the roots and stem, respectively. Furthermore, a specific and abundant occurrence of endophytic fungal strains belonging to the genus *Phyllosticta* was noted in the stem of *Ephedra sinica*. Conversely, a substantial presence of endophytic members from the genera *Aspergillus*, *Talaromyces*, and *Aporospora* was observed in the roots of the same plant. These insights contribute to a more nuanced understanding of the diverse fungal communities associated with different plant tissues. (Varma *et al.*, 2024)

Metabolomics

Metabolomics, when applied to the investigation of endophytic fungi, entails the comprehensive analysis and comprehension of the entire spectrum of small molecules (metabolites) synthesized by these fungi. This analytical approach serves as a potent means to delve into the metabolic intricacies of endophytic fungi and their intricate interactions with host plants. Metabolomics enables a thorough profiling of the diverse metabolites produced by these fungi, encompassing the identification and quantification of small molecules such as secondary metabolites, amino acids, organic acids, and other bioactive compounds.

In this domain, Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry stand as commonly employed techniques for scrutinizing the diverse array of secondary metabolites and other bioactive compounds synthesized by endophytic fungi. Metabolomics plays a pivotal role in the identification and characterization of secondary metabolites, thereby contributing to the exploration of potential pharmaceutical or agricultural applications.

An illustrative application of metabolomics involves the study of *Trichoderma* species and their effects on plant growth. Auxins produced by *Trichoderma* have been documented to enhance plant growth by alleviating the adverse impacts of stress. Additionally, secondary metabolites such as 6-pentyl- α -pyrone and harzianolide, synthesized by *Trichoderma*, contribute to the growth and development of plants, analogous to the effects of auxins. Metabolomics serves as a valuable tool for investigating how diverse environmental factors influence plant metabolism, leading to alterations in compound secretion and consequently impacting plant-microbe interactions. This scientific approach provides a nuanced understanding of the intricate metabolic dynamics governing the relationships between endophytic fungi and their host plants. (Varma *et al.*, 2024)

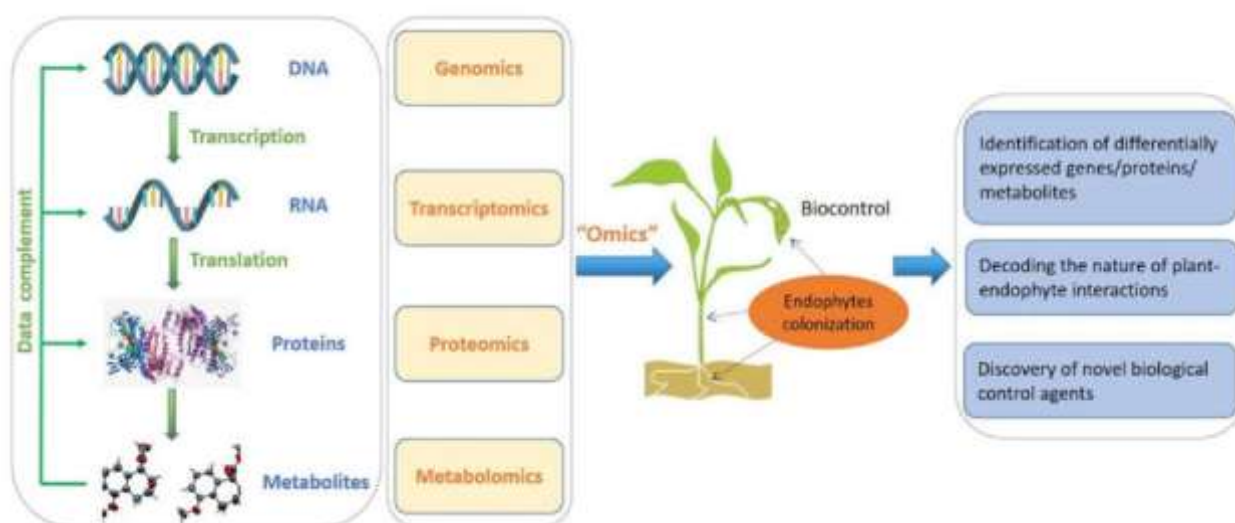


Figure: Multi-omics approaches for the research of endophytes and their metabolites in plant disease biocontrol. (Xia *et al.*, 2022)

CONCLUSION

The expeditious advancement of omics methodologies is instrumental in acquiring profound insights into the mechanisms underlying endophytic fungal strains and their effects on host plants. The integration of diverse omics tools, encompassing genetics, metagenomics, and others, plays a pivotal role in elucidating the intricate interactions characterizing the symbiotic relationships between endophytic fungal communities and their respective host plants. Omics techniques serve as comprehensive investigative tools, furnishing extensive information concerning the metabolic strategies, gene cascades, and the accumulation of key biomolecules, including metabolites, enzymes, and proteins, within endophytic fungal communities. This integrative approach allows for a more nuanced understanding of the multifaceted molecular processes governing the dynamics of endophytic fungi in association with their host plants.

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