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Isolation, Identification, and Mechanistic Analysis of Antifungal Secondary Metabolites from Endophytic Fungi in Tropical Medicinal Plants

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Abstract: The symbiotic system between tropical medicinal plants and endophytic fungi harbors a wealth of bioactive secondary metabolites. This project aims to provide potential resources for the development of novel antimicrobial drugs. Building upon previous research, the project will systematically isolate and identify secondary metabolites from endophytic fungi in tropical medicinal plants. By integrating relevant domestic and international research findings, the project seeks to clarify the core technical principles and development directions, thereby offering theoretical support and technical references for the rational utilization of endophytic fungal resources in tropical medicinal plants and the discovery of new antimicrobial active substances in China.

Keywords: tropical medicinal plants; endophytic fungi; antibacterial secondary metabolites; isolation and identification

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1. Introduction

The unique climate and ecological environment of tropical regions give rise to abundant medicinal plant resources, which form stable symbiotic relationships with endophytic fungi. Endophytic fungi, belonging to the phyla Ascomycota and Basidiomycota, are microorganisms that inhabit plants without causing visible harm to the host. They can co-evolve with their hosts and produce structurally diverse secondary metabolites with significant biological activity. These metabolites not only play crucial roles in plant defense against pathogen invasion and adaptation to environmental stress but also exhibit broad-spectrum antimicrobial activity, making them a key research focus for replacing chemical pesticides and addressing pathogen resistance issues [1].

2. Mechanism of isolation and identification of secondary metabolites with antifungal activity from endophytic fungi in tropical medicinal plants

Endophytic fungi reside within the cells or tissues of tropical plants and belong to the beneficial microbial community, which does not interfere with the normal growth of tropical medicinal plants. Currently, the majority of endophytic fungi sources in tropical plants are ascomycetes, which can persist for extended periods within shrubs, ferns, angiosperms, or herbaceous plants. Through prolonged evolution and development, tropical plants will establish a “mutualistic” coexistence relationship with endophytic fungi, enabling medicinal plants to absorb essential nutrients from the fungi. While eliminating harmful microbial populations, these endophytic fungal sources may also produce small amounts of metabolites. Technicians must prioritize the implementation of key points for the isolation and identification of antimicrobial secondary metabolites.

2.1. Host adaptation and metabolite synthesis mechanisms of endophytic fungi

Microbial-derived pesticides are favored for their residue-free nature, low resistance development potential, and compatibility with other agents. Consequently, developing novel fungicides with excellent efficacy, easy natural degradation, and no residues from microbial secondary metabolites has become a research hotspot in global pesticide innovation. The symbiotic relationship between endophytic fungi and tropical medicinal plants serves as the foundation for producing antimicrobial secondary metabolites. Endophytic fungi invade host plants through stomata or wounds, establishing stable micro-ecosystems in intercellular spaces. Through material exchange and signaling, both parties achieve co-evolution. Host plants provide nutrients such as carbohydrates and amino acids, while endophytic fungi activate specific gene clusters to synthesize secondary metabolites, enhancing host resistance to pathogenic microorganisms and forming a mutually beneficial mechanism. At the molecular level, plant endophytic fungal biosynthetic gene clusters—including polyketide synthases, nonribosomal peptide synthetases, and terpene synthases—regulate pathways for sterols, polyketides, alkaloids, and other antimicrobial substances. Their expression activity directly influences product types and yields. Endophytic fungi can also effectively inhibit the synthesis of high-concentration ethylene gas, thereby preventing the threat of high-level synthetic ethylene gas to the normal growth of tropical plants; or they can convert high-concentration ethylene gas into lower-concentration gas, achieving the purpose of stimulating the growth and development of tropical plants.

2.2. The mechanism of activity of secondary metabolites in sterilization

The antibacterial secondary metabolites produced by endophytic fungi in tropical medicinal plants have multiple antibacterial activities, which can be divided into direct and indirect mechanisms. Mainly manifested in: 1. Disrupting the structural integrity of pathogenic bacterial cells, such as sterol substances interfering with the synthesis and stability of pathogenic bacterial cell membranes, causing leakage of intracellular substances; 2. By inhibiting the metabolic pathways of pathogenic microorganisms, such as nucleic acid synthesis, protein synthesis, and key enzymes involved in energy metabolism, the growth and reproductive cycle of pathogenic bacteria can be blocked. The secondary metabolites produced by endophytic fungi can serve as signaling molecules to induce host plants to increase the expression of disease resistance related genes, thereby enhancing plant resistance to pathogens. Throughout the growth and development of tropical medicinal plants, their bodies gradually secrete specialized auxins (a type of plant hormone). This form of auxin can promote cellular development, division, and metabolism in plants. Under the influence of endophytic fungi, the frequency and quantity of auxin secretion in tropical plants can be further adjusted, which is more beneficial for the development of their cellular tissues. For instance, IAA-type plant auxins primarily originate from amino transferases, while fungi can convert these transferases into large amounts of pyruvate. Ultimately, through the combined action of oxidases and flavins, the catalytically transformed pyruvate evolves into auxins beneficial to the plant.

2.3. Molecular recognition mechanism for structural identification of metabolites

The structural analysis of secondary metabolites of endophytic fungi in tropical medicinal plants is essentially the process of accurately analyzing the molecular structure of compounds using modern analytical methods, and its core lies in the molecular recognition principles based on different analytical methods. Nuclear magnetic resonance technology can detect the nuclear spin signals of different atoms in compounds, obtain parameters such as chemical shifts and coupling constants, and infer the types, quantities, and bonding modes of functional groups in molecules, effectively distinguishing different types of compounds such as sterols and ketones. Gas chromatography-mass spectrometry and high-performance liquid chromatography utilize the differences in physical and chemical properties such as polarity and molecular weight to separate and detect target compounds. Gas chromatography-mass spectrometry is used to convert compounds into gas-phase ions, and effective ingredients such as Bis (2-ethylhexyl) phthalate are qualitatively identified based on the difference in mass to charge ratio. High performance liquid chromatography uses column chromatography separation and is combined with ultraviolet spectroscopy or mass spectrometry to achieve precise quantification and preliminary identification of trace metabolites.

3. Isolation and identification pathway of secondary metabolites from endophytic fungi in tropical medicinal plants for sterilization

3.1. Efficient isolation and precise identification of endophytic fungi

The efficient isolation and precise identification of endophytic fungi in plants are the foundation of subsequent research and require standardized technical processes. Firstly, strictly regulate the collection and pretreatment of plant samples, select healthy tropical medicinal plant tissues, and remove surface attached bacteria through gradient surface sterilization and other methods to ensure that the isolated endophytic fungi come from within the plant. Secondly, based on the differences in nutritional requirements of endophytic fungi, suitable culture media are screened, and the composition of the culture medium is optimized by adding host plant tissue extracts and other methods to improve its isolation rate. To prevent contamination by miscellaneous bacteria, the strain was purified using the mycelial top purification method. In terms of strain identification, a combination of morphological and molecular identification methods is used for identification [2]. Tropical plants survive in harsh natural environments for a long time. Throughout their entire growth cycle, tropical plants are susceptible to factors such as high salt soils, dry and low rainfall climates, pathogens, heavy metals, etc. Endophytic fungi can indirectly regulate the growth environment of plants by changing the frequency and quantity of hormones secreted by the plant body. Based on this, technicians can use the method of isolating fungi in plants to identify the properties and fission patterns of fungi stored in tropical medicinal plants, and summarize the common secondary metabolites of fungi in plants. The above approach can provide strong support for the study of metabolites in tropical medicinal plants.

3.2. Optimization of fermentation conditions and induction of metabolite synthesis

The key to isolating and identifying this strain is to improve the production of secondary metabolites of antibacterial activity by optimizing fermentation conditions and constructing an induction system. Firstly, choose an appropriate fermentation mode. Liquid deep fermentation has the advantages of fast growth and no environmental pollution, making it suitable for large-scale production of mycelium and metabolites; Solid state fermentation can simulate the growth environment of host plants and facilitate the synthesis of specific metabolites. Secondly, through single factor experiments and response surface methodology, the temperature pH. Optimize key parameters such as ventilation time and carbon nitrogen source ratio to maximize the yield of the target product. For example, adjusting the types of carbon and nitrogen in the culture medium has a significant impact on the synthesis of secondary metabolites in endophytic fungi. On this basis, an efficient induction system is constructed, which activates silenced gene clusters by adding host plant extracts, signaling molecules, microbial elicitors, and other means, and induces the production of new antibacterial compounds through co culture with host plant cells. After fermentation, centrifuge and filter the fermentation broth to prepare for subsequent extraction and purification.

3.3. Multi step separation and purification, as well as enrichment of target products

By utilizing multi-stage cascade separation and purification technology, appropriate process combinations are selected based on the physicochemical properties of metabolic products to achieve efficient separation and enrichment of target products. Firstly, solvent extraction is used to preliminarily separate the fermentation broth or mycelium. By utilizing the polarity differences of solvents with different polarities, gradient extraction method is used to remove a large amount of water-soluble or lipophilic impurities and enrich the target product. Secondly, using column chromatography, lipid soluble components are separated by silica gel column chromatography, and the polarity gradient of the eluent is adjusted to achieve the separation of different components. Gel column chromatography is based on the difference of molecular size to achieve separation, suitable for the removal of small molecular impurities, can also achieve the preliminary separation of similar compounds. For target products with high purity requirements, high-performance liquid chromatography is used for purification. Select suitable chromatographic columns and mobile phase systems, and optimize parameters such as flow rate, column temperature, and detection wavelength to achieve precise separation and collection of target products. At the same time, thin-layer chromatography is used to rapidly detect the target product, track the elution position of the target product, and improve separation efficiency [3]. When necessary, technicians can also jointly use gas chromatography, liquid chromatography, gel column chromatography and other detection methods to narrow the gap between the detection data and the real situation. Technicians also need to accurately evaluate the types and quantities of fungi present in tropical medicinal plants by observing the separation and changes of the target product, record various experimental data, use high-power microscopes to observe the enrichment status of the target product, and finally compare the mechanism of action of secondary metabolites from endophytic fungal sources in plant sterilization.

3.4. Multi technology collaborative application of structural identification

This project aims to establish a multi technology collaborative structural identification system to ensure the accuracy and completeness of metabolic product structures. Firstly, by using high-performance liquid chromatography combined with UV visible spectroscopy, the retention time and maximum absorption wavelength of the target product in UV visible spectroscopy were obtained, and the type of compound was preliminarily determined. Secondly, GC-MS, LC-MS and other techniques are used to perform mass spectrometry analysis on its molecular weight, fragment ions, etc., to infer its molecular formula and basic structure, such as using GC-MS, etc; Determine its molecular composition; Finally, the molecular composition was determined using gas chromatography-mass spectrometry (GC-MS) technology. For complex compounds, various spectroscopic methods such as 1H-NMR, 13C-NMR, DEPT, COSY, HSQC, HMBC, etc. are used to determine the bonding mode of each atom, the position of functional groups, and the structure of carbon hydrogen bonds in the compound. For chiral compounds, circular dichroism was used to determine their stereoisomers by analyzing the circular dichroism data.

3.5. Systematic analysis and activity verification of the mechanism of action

On the basis of preliminary work, enterprises need to adopt interdisciplinary approaches to systematically analyze the mechanism of action of antibacterial secondary metabolites and conduct systematic activity verification. Firstly, through in vitro antibacterial experiments, the inhibitory effect of the target product on different pathogenic bacteria is determined, and the antibacterial activity and strength of the target product are clarified. Secondly, through microscopic observation such as scanning electron microscopy, transmission electron microscopy, etc., the influence of metabolites on the cell structure of pathogenic bacteria is analyzed to explore their mechanism of action. Finally, using fluorescence quantitative PCR and other techniques, the influence of metabolites on the cell structure of pathogenic fungi was explored. Enzyme activity measurement, real-time fluorescence quantitative PCR and other techniques were used to detect the activity of key enzymes involved in pathogenic fungi metabolism and changes in the expression levels of disease resistance related genes, revealing the regulatory mechanism of metabolites on pathogenic fungal metabolic pathways and signaling pathways. Predict its target genes through bioinformatics and validate them through in vitro experiments.

4. Conclusion

In summary, the research results of this project will provide new ideas and methods for the development of secondary metabolites of endophytic fungi in tropical medicinal plants. In terms of application, it is necessary to conduct in-depth research on its mechanism of action and combine cutting-edge technologies such as molecular biology and bioinformatics to clarify its target and regulatory network.

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Disclosure statement

The author declares no conflict of interest.

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