

DOCTORAL DISSERTATION ABSTRACT

Title: *Extraction and evaluation of the prebiotic activity of polysaccharide derived from mushroom mycelia (Cordyceps militaris and Trametes versicolor) and their biomass application in food supplement.*

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Executive summary:

1. Rationale for the study

In recent years, the rapid increase in non-communicable chronic diseases associated with metabolic syndrome, including diabetes mellitus, obesity, gout, and gastrointestinal disorders, has intensified scientific interest in the role of the gut microbiota in maintaining human health.

Prebiotics serve as selective substrates that promote the growth and metabolic activity of beneficial microorganisms (probiotics), thereby contributing to the establishment and maintenance of a balanced intestinal microbial ecosystem. More importantly, the fermentation of prebiotics by probiotic bacteria leads to the production of short-chain fatty acids (SCFAs), which play critical physiological roles in colon health by providing energy

for colonocytes, enhancing mineral absorption, regulating intestinal pH, and reducing the risk of colorectal cancer, inflammatory bowel diseases, and other gastrointestinal disorders.

Given the substantial health benefits associated with prebiotics, current research efforts have increasingly focused on identifying novel sources of prebiotic compounds with high production potential and superior biological safety. In this context, mushrooms have emerged as promising candidates due to their abundance of non-digestible carbohydrates, including chitin, hemicellulose, β - and α -glucans, mannans, xylans, and galactans. These polysaccharides, together with low-molecular-weight carbohydrates such as glucose, galactose, fructose, and N-acetylglucosamine, can function as specific substrates that selectively stimulate the growth and activity of beneficial gut microorganisms, particularly members of the genera *Bifidobacterium* and *Lactobacillus*.

Mushroom-derived polysaccharides, particularly those obtained from *Cordyceps militaris* and *Trametes versicolor*, possess highly stable molecular structures that resist digestion in the upper gastrointestinal tract and exhibit remarkable selectivity in stimulating the growth of beneficial gut microorganisms, especially *Lactobacillus* and *Bifidobacterium* species. *C. militaris* has long been recognized in traditional medicine for its health-promoting properties, including enhancement of vitality, support of respiratory function, and immunomodulatory effects. Meanwhile, *T. versicolor* is widely regarded as a rich source of β -glucans and biologically active polysaccharide-peptide complexes, such as polysaccharopeptide (PSP) and polysaccharide-K (PSK), which have been extensively utilized as adjuvants in cancer therapy and immune enhancement.

Despite their recognized biological value, the commercial exploitation and utilization of these two mushroom species remain largely focused on fruiting bodies. Conventional fruiting-body cultivation on solid substrates is generally time-consuming, highly dependent on environmental conditions, difficult to standardize in terms of product quality, and associated with relatively high production costs. These limitations present significant challenges for large-scale industrial applications.

To address these constraints, the production of fungal mycelial biomass through submerged fermentation (SmF) has emerged as a promising alternative. Compared with traditional fruiting-body cultivation and solid-state fermentation (SSF), SmF offers several advantages, including substantially shorter cultivation cycles, precise control of environmental parameters such as pH, temperature, and dissolved oxygen, as well as greater suitability for industrial-scale production. Moreover, numerous studies have demonstrated

that submerged-cultivated mycelia not only achieve high biomass yields but also accumulate substantial amounts of bioactive polysaccharides with distinctive functional properties.

Nevertheless, most existing studies have primarily focused on the extraction and characterization of bioactive compounds from fruiting bodies, whereas the prebiotic potential of polysaccharides derived from submerged-cultivated fungal mycelia remains largely underexplored. In light of these practical considerations and scientific perspectives, the present study, entitled “*Extraction and evaluation of the prebiotic activity of polysaccharide derived from mushroom mycelia (Cordyceps militaris and Trametes versicolor) and their biomass application in food supplement.*”, was undertaken.

2. Research objectives

2.1. General objective

To establish a scientific basis for the cultivation conditions, morphological characteristics, and biological activities (antioxidant and prebiotic properties) of polysaccharide fractions derived from the mycelia of *Cordyceps militaris* and *Trametes versicolor*, and to evaluate the potential application of fungal biomass in the development of biologically active fermented functional beverages.

2.2. Specific objectives

To optimize submerged cultivation conditions for maximizing biomass production and polysaccharide yield from *Cordyceps militaris* and *Trametes versicolor* mycelia.

To evaluate and compare the extraction yields, morphological characteristics, and biological activities (prebiotic and antioxidant properties) of polysaccharide fractions sequentially extracted using hot water extraction (HWE), alkaline extraction (AE), and acid extraction (AEc).

To investigate the potential application of fungal mycelial biomass in the production of a lactic acid-fermented oyster mushroom beverage enriched with probiotic microorganisms and possessing health-promoting biological activities, including antioxidant and antimicrobial properties.

3. Scope and research subjects

3.1. Research subjects

The fungal mycelia of two mushroom species were selected as the primary research subjects: *Cordyceps militaris* and *Trametes versicolor*.

3.2. Scope of the study

The scope of this dissertation is limited to the following aspects:

- The study focuses on two fungal species, *Cordyceps militaris* and *Trametes versicolor*, with particular emphasis on the biological activities of mycelium-derived polysaccharides and the application potential of fungal mycelial biomass.
- Three different polysaccharide fractions, namely hot water-extracted polysaccharides (HWE), alkali-extracted polysaccharides (AE), and acid-extracted polysaccharides (AEc), were isolated from the mycelia of *Cordyceps militaris* and *Trametes versicolor* and evaluated for their prebiotic potential.
- The probiotic microorganisms used for prebiotic activity assessment included *Lactiplantibacillus plantarum* WCFS1, *Lacticaseibacillus casei* 01, *Bifidobacterium animalis* YC381, *Lactiplantibacillus pentosus* NH1, and *Pediococcus acidilactici* NBD8. Pathogenic bacteria employed for comparative evaluation included *Escherichia coli* ATCC 85922 and *Staphylococcus aureus* ATCC 25023.
- Fruiting bodies of oyster mushroom (*Pleurotus ostreatus*) were obtained from Quynh Tam Mushroom Farm, Hoa Phong Commune, Hoa Vang District, Da Nang City, Vietnam, and were used as raw materials for the production of a fermented mushroom beverage.
- Experimental work was conducted at the laboratories of the Faculty of Biology, Agriculture and Environmental Science, University of Science and Education – The University of Danang, and the Faculty of Chemistry, University of Science and Technology – The University of Danang.
- The research was carried out during the period from 2021 to 2025.

4. Research methodology

4.1. Theoretical research methods

Relevant scientific literature, including books, journal articles, theses, dissertations, and research reports, was systematically collected and reviewed. The literature survey focused on fungal cultivation and optimization of mycelial growth conditions, extraction and fractionation of polysaccharides from plant and fungal sources, evaluation of antioxidant and prebiotic activities of polysaccharides and other prebiotic compounds, as well as the development of fermented food products. Both domestic and international publications were consulted to establish the scientific foundation of the study.

4.2. Experimental research methods

Optimization of cultivation conditions for the production of polysaccharide-rich fungal biomass:

- Investigation of the effects of cultivation parameters on mycelial growth under submerged fermentation conditions.
- Determination of the optimal cultivation conditions for maximizing fungal biomass production and polysaccharide accumulation.

Sequential extraction and characterization of polysaccharide fractions:

- Sequential extraction of polysaccharides using hot water extraction (HWE), alkaline extraction (AE), and acid extraction (AEc).
- Evaluation of extraction yields, morphological characteristics, antioxidant activities, and prebiotic properties of the obtained polysaccharide fractions.

Application of fungal mycelial biomass in functional food development:

Assessment of the feasibility of incorporating fungal mycelial biomass into the formulation of value-added functional food products, particularly probiotic fermented beverages.

Data collection and statistical analysis: Experimental data were systematically collected, processed, and statistically analyzed to evaluate the effects of cultivation conditions and the biological activities of the extracted polysaccharides, as well as to validate the reliability and significance of the obtained results.

5. Research contents

Content 1: Optimization of submerged cultivation conditions for the production of fungal biomass and polysaccharides

Content 2: Sequential extraction and evaluation of morphological characteristics, antioxidant activity, and prebiotic potential of polysaccharide fractions

Content 3: Application of fungal mycelial biomass in functional food development

6. Scientific and practical significance of the dissertation

6.1. Scientific significance

The dissertation provides a comprehensive and systematic scientific dataset on the effects of nutritional factors (carbon and nitrogen sources) and environmental conditions (pH) on biomass production and polysaccharide accumulation in the mycelia of *Cordyceps militaris* and *Trametes versicolor* under submerged fermentation (SmF) conditions.

The dissertation demonstrates the relationship between extraction methods and biological activities. A comparative analysis of sequentially extracted polysaccharide fractions (water-, alkali-, and acid-extracted fractions) was conducted. The results showed that different polysaccharide fractions exhibited different antioxidant activities and different prebiotic activities toward different probiotic strains. These findings contribute a new

perspective on the selection of extraction methods for function-oriented product development, rather than focusing solely on conventional hot-water extraction.

The research provides specific quantitative evidence regarding digestion resistance (α -amylase resistance), in vitro prebiotic index (PI), and particularly the production of short-chain fatty acids (SCFAs; acetate, propionate, and butyrate) from mushroom polysaccharides fermented by specific probiotic strains (*Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Bifidobacterium animalis*, *Lactiplantibacillus pentosus*, and *Pediococcus acidilactici*). These findings contribute to strengthening the theoretical basis for the use of medicinal mushroom mycelia as a source of prebiotics.

The dissertation provides a scientific foundation for further studies on the molecular properties, chemical structures, and biological interactions of polysaccharides with the gut ecosystem, as well as for the discovery of novel polysaccharide molecules from fungal mycelia.

6.2. Practical significance

Optimal conditions for the submerged cultivation of *C. militaris* and *T. versicolor* were established to achieve high biomass yield and polysaccharide content. This represents a feasible technological solution for producing organic fungal biomass, helping to ensure a stable supply of raw materials for the food and pharmaceutical industries. In addition, submerged cultivation serves as a rapid propagation method, improving mushroom production efficiency and modernizing mushroom cultivation technology.

The study contributes to the diversification of mushroom-based health-promoting food products. The application-oriented research resulted in the development of a “Fermented Oyster Mushroom Beverage Supplemented with *Trametes versicolor* Mycelia,” which exhibited good sensory quality, complied with food safety and hygiene requirements, and possessed verified biological activities (antibacterial and prebiotic activities). This product represents a practical model with commercialization potential and meets current consumer demands for green foods and gut health-supporting products.

7. Dissertation structure

The dissertation comprises 134 pages (excluding appendices and references) and is organized as follows:

The **Introduction**, consisting of 5 pages, presents the research rationale, objectives, research contents, research methodology, scientific significance, and practical significance of the dissertation.

The main body of the dissertation is divided into three chapters:

- Chapter 1. Literature Review – 38 pages;
- Chapter 2. Materials and Methods – 22 pages;
- Chapter 3. Results and Discussion – 63 pages.

The Conclusions and Recommendations section consists of 3 pages, while the List of Published Research Outputs occupies 2 pages.

In addition, the References section comprises 23 pages. The dissertation contains a total of 24 tables and 30 figures and graphs. A total of 223 references, including both English and Vietnamese sources, were cited. These references consist of scientific journal articles and academic books.

8. Novel points of the doctoral dissertation

** In terms of academic:*

Systematization of scientific knowledge on submerged cultivation technology: The dissertation established several optimal technological conditions for the submerged cultivation of *Cordyceps militaris* and *Trametes versicolor* to promote the biosynthesis of polysaccharide-rich fungal biomass. These findings provide an important scientific basis for elucidating the effects of environmental factors (pH, carbon sources, and nitrogen sources) on biomass accumulation and intracellular polysaccharide production in fungal cells.

Elucidation of the effectiveness of sequential extraction techniques: The study demonstrated the superiority of sequential extraction methods in fractionating polysaccharides according to their structural characteristics and solubility properties. A notable novel finding is that the acid-extracted polysaccharide fraction (A-PS), which has received limited attention in previous studies, exhibited the most promising prebiotic activity, as evidenced by the highest Prebiotic Index (PI) values for several probiotic strains. This discovery opens a new avenue for exploiting tightly bound polysaccharides present in fungal cell walls.

Providing a scientific foundation for the discovery of novel bioactive polysaccharides: The research outcomes establish a foundation for further purification, isolation, and identification of novel polysaccharides derived from fungal mycelial biomass, particularly those possessing distinctive antioxidant and prebiotic properties.

Confirmation of the prebiotic potential of fungal mycelial biomass: The dissertation provides reliable experimental evidence demonstrating that fungal mycelial biomass functions as an effective prebiotic substrate. It selectively stimulates the growth of beneficial microorganisms, including *Lactobacillus* spp. and *Bifidobacterium* spp., while inhibiting pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* through the

production of short-chain fatty acids (acetic, propionic, and butyric acids).

** In terms of practical:*

Proposing a technological solution for sustainable raw material production: The dissertation demonstrated the feasibility of submerged cultivation for producing fungal mycelial biomass as a valuable source of organic raw materials for food and pharmaceutical applications. This approach offers several advantages, including stable production, high productivity, short cultivation cycles, ease of industrial-scale implementation, controllable cultivation conditions, and compatibility with automation systems. These findings provide an important foundation for diversifying raw material production models beyond traditional fruiting-body cultivation.

Diversification of prebiotic sources for industrial applications: While commercially available prebiotics are currently derived primarily from plant sources (e.g., fructooligosaccharides and inulin) and marine algae (e.g., fucoidan and carrageenan), the results of this dissertation indicate that fungal mycelial biomass represents a promising alternative source of prebiotics. Furthermore, cultivation conditions can be precisely controlled to facilitate large-scale industrial production.

Successful development of a probiotic-rich fermented beverage with enhanced antioxidant activity: The dissertation successfully developed a technological process for producing a fermented beverage from *Pleurotus* mushrooms supplemented with *Trametes versicolor* mycelial biomass. The resulting product not only satisfied food safety requirements but also demonstrated the successful integration of prebiotic components (fungal mycelia) and probiotic microorganisms (fermentative bacteria) to create a high-value functional food product with strong potential for technology transfer and commercial application..

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Doctoral candidate

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