MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Comparative study on the mycelial fermentation of a wild type and domesticated cultivation type of *Ganoderma lucidum***
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
Yang SHIHAN

Scientific supervisor
Ihor HRETSKYI, Ph.D., As. prof.

Reviewer
Olga IUNGIN, Ph.D., As. prof.

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APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA

_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Yang Shihan

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Scientific supervisor Ihor Hretskyi, Ph.D., As. prof

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3. Content of the thesis (list of questions to be developed): literature review; object, experimental part; conclusions

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I am familiar with the task:

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SUMMARY

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Ganoderma lucidum is a traditional and important medicinal fungus with a long history. It has attracted attention due to its bioactive components such as *Ganoderma* polysaccharides and triterpenes, and is known for its potential effects in regulating intestinal flora, lowering blood sugar, and exhibiting anticancer properties. The limited quantity of wild *Ganoderma lucidum* cannot meet the daily needs of people or the demands of the industry. Therefore, the artificial cultivation *of Ganoderma* This study investigates the fermentation characteristics of wild and cultivated *Ganoderma lucidum* mycelia through comparative analysis. The differences in fermentation process between the two types were explored by utilizing different culture media and fermentation conditions, focusing on parameters such as growth rate, enzyme production capability, and content of bioactive components. Results indicate notable disparities between wild and cultivated strains during fermentation, including variations in growth rate, enzyme production, and content of bioactive constituents. Additionally, analysis of fermentation products revealed differences in medicinal value and nutritional composition between the two types. This research provides a theoretical basis for the production and utilization of *Ganoderma lucidum,* offering valuable insights for further investigation into its fermentation characteristics and optimization of production processes.

Cultivated Ganoderma.

TABLE OF CONTENTS

INTRODUCTION

Ganoderma lucidum has antioxidant, anti-tumor, anti-aging, and liver-protective functions. However, wild *Ganoderma lucidum* is too rare to be commercially viable, making it crucial to increase its production. While artificial cultivation can expand production, it faces issues of instability and differences from wild *Ganoderma lucidum.* This study aims to better understand the differences between artificially cultivated liquid fermentation mycelium of *Ganoderma lucidum* and wild *Ganoderma lucidum,* and to overcome these differences to provide insights for commercialization and enhance the production of active ingredients in artificial *Ganoderma* mycelium. By studying the growth characteristics and metabolic products of mycelium fermentation, exploring the differences between wild and cultivated strains, optimizing production processes, analyzing the medicinal components and pharmacological effects of mycelium, and investigating differences in environmental adaptability and stress resistance, this research aims to provide scientific basis for evaluating the medicinal value of *Ganoderma lucidum* and offer theoretical guidance for its protection and cultivation. Through the study of wild and artificially cultivated mycelium, efforts will be made to overcome the low yield of wild *Ganoderma lucidum*, identify differences between the two, continuously optimize artificial Ganoderma fermentation methods, and enhance the bioactive compounds in *Ganoderma lucidum*.

Purpose of the study:

1. To understand the differences between artificially cultivated liquid fermented *Ganoderma mycelium* and wild *Ganoderma,* and further overcome these differences, providing references for the commercialization of *Ganoderma.*

2. Study the growth characteristics and metabolic products during the mycelium fermentation process, explore the differences between wild and cultivated strains in the mycelium fermentation process, contributing to the optimization of Ganoderma production processes.

3. Overcome the low yield characteristic of wild Ganoderma through the study of wild and artificially fermented mycelium, identify the differences between the two, continuously optimize the method of artificial *Ganoderma* fermentation, and enhance the bioactive substances in *Ganoderma*.

Object of study:

1.Ganoderma is a very precious traditional Chinese medicine that has a history of more than 2,000 years. It belongs to the polypore fungi family and grows on the decayed wood of broad-leaved trees. *Ganoderma* contains a variety of bioactive components, including Ganoderma polysaccharides and Ganoderma triterpenes.

2. *Ganoderma lucidum* has the effects of regulating intestinal flora, antioxidation, anti-cancer, lowering blood sugar, and lowering blood lipid levels.

Subject of study:

1. Strain identification and comparison require first identifying the wild-type and domesticated Ganoderma lucidum strains to determine their taxonomical information and genetic traits.

2. Then, we need to compare the two strains in terms of morphological characteristics, growth rate, metabolic properties, and so on.

CHAPTER 1
LITERATURE REVIEW

1.1 Overview and Research Progress of Ganoderma

1.1.1 Overview of Ganoderma

Ganoderma is a very precious traditional Chinese medicine, belonging to the fungi kingdom and the polyporaceae family [1]. It is a large medicinal fungus that commonly grows on decayed wood of broad-leaved trees. Its cap is semi-umbrella-shaped, red on the top, and white on the bottom, with a lacquer-like gloss [2]. Ganoderma has many beneficial effects on human health and has been considered to have anti-aging effects in ancient Chinese medical books such as the "Shennong's Classic of Materia Medica" and the "Compendium of Materia Medica"[3]. In the Chinese Pharmacopoeia, Ganoderma's functions include restoring biological energy, relaxing the mind, and relieving coughs and asthma. In ancient times, *Ganoderma* was also known as the "herb of immortality"[4]. In the past century, due to its medicinal and cultural value, *Ganoderma* has been widely used in clinical medicine in Asia, with significant economic value.

1.1.2 The medicinal components of Ganoderma

Ganoderma and its active components have various effects such as regulating blood lipids, lowering blood sugar, antioxidation, and anti-aging. The fruiting body of *Ganoderma* is rich in a variety of essential vitamins, active amino acids, polysaccharides, triterpenes, and other bioactive peptides with anti-tumor properties, with polysaccharides and triterpenes being particularly noteworthy for their important roles [5]. *Ganoderma* has regulatory effects on the nervous system [6], can reduce central nervous system excitability [7], has analgesic effects [8], detoxifying properties [9], anti-radiation effects [10], enhances immune function [11], and has therapeutic effects on asthma. In recent years, *Ganoderma* has been internationally recognized as an effective adjuvant in cancer treatment.

Anti-cancer activity: Clinical studies have shown that *Ganoderma,* as a

traditional Chinese medicine, has good efficacy in adjuvant therapy without significant toxicity [12]. *Ganoderma* is recognized as a natural Chinese medicine product, and *Ganoderma* polysaccharides (GLP) can inhibit cancer through immune regulation, anti-proliferation, promoting apoptosis, inhibiting metastasis, and anti-angiogenesis effects. It activates immune cells, enhances phagocytosis, boosts cytokine production, and enhances natural killer (NK) cell toxicity, thus preventing and treating various cancers such as breast cancer, prostate cancer, colon cancer, and cervical cancer [13].

Modulation of Intestinal Microbiota: The human intestines have a large surface area, playing a crucial role not only in digesting and absorbing nutrients but also in protecting against intestinal pathogens. Imbalances in the intestinal microbiota can lead to damage to the intestinal barrier, disrupt host endogenous metabolites, and consequently harm the body, leading to conditions such as obesity and hypertension. Many components in Ganoderma have a regulatory effect on the intestinal microbiota, with positive impacts. Extracted polysaccharides and lipophilic components from Ganoderma spores can alter the biological structure of intestinal microorganisms, improve normal intestinal flora, and regulate the control of intestinal microbiota and fat metabolism [14].

Improvement in Type 2 Diabetes: Diabetes increasingly threatens people's health. Natural plants and fungi have different mechanisms for their anti-diabetic effects: increasing insulin release, reducing glucose absorption in the intestines, increasing glycogen synthesis, and enhancing this pathway. *Ganoderma* polysaccharides (GLP) can inhibit the activity of α-glucosidase, have a regulatory effect on the intestinal microbiota related to type 2 diabetes, and play an important role in improving type 2 diabetes.

Anti-inflammatory effect: The intestinal microbiota can effectively utilize bioactive components in natural substances such as herbal medicines, enhancing the digestive efficiency of the intestinal microbiota. Ganoderma triterpenes have anti-inflammatory activity, and Ganoderma water extract, as a prebiotic, can increase the composition of beneficial gut microbiota and improve low-grade chronic

inflammation in mice to alleviate obesity [15]. According to studies, short-chain fatty acids (SCFs) can enhance IL-10 production and inhibit the generation of pro-inflammatory cytokines, thus improving inflammation.

Antioxidant effect: Reactive oxygen or nitrogen compounds produced in the body can lead to many diseases, such as Alzheimer's disease and oxidative damage associated with cancer. *Ganoderma* may become a natural, non-toxic antioxidant resource in the future. *Ganoderma* triterpenes have antioxidant properties and reduce oxidative stress by clearing free radicals produced in cells. *Ganoderma* polysaccharides (GLP) not only have a strong quenching effect on superoxide anions generated by the self-oxidation of benzene triphenyl, but also exhibit a certain dose-dependent effect.

Improvement in obesity: The diversity of gut microbiota in obese individuals is lower compared to non-obese individuals. Treating obesity with natural components is a relatively safe method. *Ganoderma* can regulate the composition of gut microbiota, improve obesity induced by a high-fat diet (HFD), hyperlipidemia, and hepatic steatosis. GA in *Ganoderma* can effectively alleviate lipid metabolism disorders and improve the imbalance of gut microbiota, influencing the production of metabolites by certain gut microbiota to achieve regulatory effects, mainly by regulating the biosynthesis of functional fatty acids in the liver.

Antibacterial effect: *Ganoderma,* combined with other drugs, can be used to treat various bacterial diseases. *Ganoderma* polysaccharides have effective inhibitory effects on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli, Salmonella*, and other bacteria, although the inhibitory effect weakens under acidic conditions. Fermentation liquids of *Ganoderma* fungi also have different inhibitory effects on various types of microorganisms, with different fermentation liquids having different effects. The acidic components of *Ganoderma* also exhibit antibacterial properties.

1.1.3 The growth environment and distribution of *Ganoderma.*

Ganoderma, a precious medicinal fungus, mainly grows in temperate and tropical regions with broad-leaved forests, commonly found in Asia, North America,

and Europe. It typically thrives on the trunks or roots of trees, especially on hardwood trees such as oak, maple, and beech. *Ganoderma* has strict requirements for its growth environment, needing suitable temperature, humidity, and light conditions [16]. Generally, it thrives in warm and humid environments, with the optimal temperature for growth being 20-30 degrees Celsius and humidity above 70%. Additionally, *Ganoderma* also requires adequate light, but it should not be directly exposed to sunlight. In China, *Ganoderma* is mainly distributed in the southern, central, and eastern regions, such as Guangdong, Guangxi, Fujian, Hunan, and Zhejiang. In the wild, *Ganoderma* is often concealed under the trunks or roots of trees, making it relatively difficult to find. In recent years, due to the widely recognized medicinal value of *Ganoderma,* artificially cultivated *Ganoderma* has been increasing.

1.1.4 Current Status of Ganoderma Development

For hundreds of years, the miraculous therapeutic effects and beneficial health properties of mushrooms have gained attention from people worldwide [17]. *Ganoderma,* known as the "King of Herbs" for its effects in lowering blood sugar, detoxifying, and anti-tumor properties, has been discovered in 104 species, with approximately 40 species being used for medicinal purposes. Among them, red *Ganoderma*, purple *Ganoderma*, and *Ganoderma tsugae* are included in the Chinese Pharmacopoeia. Wild Ganoderma is rare, and in ancient times, only a few individuals could consume it; nowadays, *Ganoderma* is mostly artificially cultivated and is recognized as a natural product that benefits the immune system and improves overall health [18]. The modern pursuit of health and the continuous discovery of the medicinal value of *Ganoderma* have promoted the development of artificially cultivated *Ganoderma*, leading to an increasing demand for *Ganoderma* [19].

1.2 Differences Between Wild and Cultivated Ganoderma

1.2.1 Growth Environment

Artificially cultivated *Ganoderma* typically grows in controlled environments

where factors such as temperature, humidity, and light are regulated to promote growth. In contrast, wild *Ganoderma* grows in natural environments, often on trees in forests and mountainous areas, commonly on broad-leaved trees. For example, purple *Ganoderma* grows on fallen or decayed logs in tropical forests, while brown *Ganoderma* grows on decayed wood stumps of broad-leaved trees [20].

1.2.2 Appearance Characteristics

Artificially cultivated Ganoderma usually has a more uniform appearance in terms of shape and color. In contrast, wild Ganoderma may exhibit more variations in shape, color, and size. Wild Ganoderma typically has a fan or umbrella-like shape with a smooth and glossy surface, displaying various colors such as red, orange, yellow, purple, often with black spots or stripes. Sometimes, it may have pore-like structures resembling "eyes" on the surface, which are spore-producing organs. The edges of Ganoderma are wavy or toothed, with a regular overall shape. The texture of Ganoderma is hard, sometimes slightly elastic, with a milky white or pale yellow cross-section, and occasionally showing patterns. Overall, wild Ganoderma has an aesthetically pleasing appearance, unique form, and is an ancient and miraculous medicinal fungus.

1.2.3 Medicinal Value

It is generally believed that wild Ganoderma has higher medicinal value because it grows in natural environments, potentially containing more active ingredients. Conversely, artificially cultivated Ganoderma may be influenced by external factors during the cultivation process, leading to relatively lower medicinal value. Both wild and cultivated Ganoderma have unique characteristics in terms of medicinal value:

Medicinal Value of Wild Ganoderma:

Natural Environment: Wild Ganoderma grows in natural environments, absorbing abundant natural nutrients and trace elements, making it rich in medicinal components and considered to have higher medicinal value.

Diversity: The medicinal components of wild Ganoderma may vary due to different growth environments, resulting in greater diversity and active ingredients.

Rarity: Wild Ganoderma is usually rare, thus possessing certain value for collection and research.

Medicinal Value of Cultivated Ganoderma:

Control: The growth environment of cultivated Ganoderma can be strictly controlled, resulting in more stable and controllable yields and medicinal components.

Large-scale Production: Cultivated Ganoderma can be produced on a large scale to meet market demand, with the potential to increase the yield of medicinal components through optimized cultivation conditions.

Sustainability: Due to the limited resources of wild Ganoderma, cultivated Ganoderma enables the sustainable utilization and protection of wild Ganoderma resources.

In summary, both wild and cultivated Ganoderma have their own advantages and disadvantages in terms of medicinal value. Wild Ganoderma offers advantages in terms of ecological authenticity and diversity, while cultivated Ganoderma provides controllability and sustainable utilization. Therefore, they complement each other and contribute to human health.

1.2.4 Price and Supply

Due to the relative difficulty in collecting wild Ganoderma and its limited availability, the price is higher and the supply is unstable. In contrast, artificially cultivated Ganoderma can meet market demand through large-scale cultivation, resulting in a relatively lower price and more stable supply.

1.3 Mycelial Fermentation Methods

1.3.1 Solid-state Fermentation

Solid-state fermentation involves cultivating Ganoderma mycelium in solid substrates such as rice husks and corn stover, providing a suitable culture medium.

During solid-state fermentation, the mycelium grows in the solid substrate and produces beneficial compounds. This method is suitable for specific Ganoderma fungal strains and can improve the quality and purity of the products.

1.3.2 Submerged Fermentation

Ganoderma submerged fermentation involves cultivating Ganoderma mycelium in liquid culture media to promote fermentation. The fungus is inoculated into the liquid culture medium, allowing it to grow and ferment [21]. The liquid culture medium typically includes carbon sources, nitrogen sources, mineral salts, and through appropriate control conditions such as temperature, pH, and oxygen supply, the mycelium undergoes fermentation.

1.3.3 Mixed Fermentation

Mixed fermentation involves fermenting Ganoderma mycelium together with other microorganisms, taking advantage of the interactions between different microorganisms to increase the yield and quality of the products. It can be achieved by adjusting the proportions of different microorganisms and fermentation conditions.

1.3.4 Complementary Fermentation

Complementary fermentation involves fermenting different species of Ganoderma mycelium together to obtain a richer combination of products. Different species of Ganoderma fungi may contain different active ingredients, and complementary fermentation can yield a greater variety of beneficial components.

1.3.5 Dual-directional Fermentation Technology in Traditional Chinese Medicine

Dual-directional fermentation technology utilizes medicinal fungi such as Ganoderma as the fermentation strain and combines it with active ingredients from traditional Chinese medicinal materials as the fermentation substrate [22]. The medicinal materials not only provide nutrients but also allow the fungal enzymes to

interact with and alter their important components, making the fermentation process bidirectional [23, 24]. For example, adding traditional Chinese medicinal materials such as Chinese yam, Angelica sinensis, and coix seeds during Ganoderma fermentation can enhance the level of active substances produced by Ganoderma and promote the generation of active materials [25].

1.4 Research Objectives and Significance

1.4.1 Main Research Content

Strain Identification and Comparison: Initially, it is necessary to identify and compare wild and cultivated Ganoderma strains to determine their taxonomic information and genetic characteristics, comparing their morphological features, growth rates, and metabolic properties.

Comparison of Fermentation Conditions: Comparing the growth of wild and cultivated

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Experimental Materials and Instruments

2.1.1 Main Materials

A wild fungal fruiting body collected in 2020 from Luoshan Scenic Area in Yantai, Shandong. (Font size: 12, line spacing: 1.5, same for the following text.)

2.1.2 Experimental Reagents and Instruments

Centrifuge (Eppendorf), PCR machine (ABI), electrophoresis apparatus (Beijing Liuyi Instrument Factory), water bath (Shanghai Jinghong Experimental Equipment Co., Ltd.), BGI 2xSuper PCR lMix (with dye) (Beijing Lihe Huada Genetics Technology Co., Ltd.), BGID2000 Plus DNA ladder (Beijing Lihe Huada Genetics Technology Co., Ltd.), agarose (Solarbio), fungal genome extraction kit (Tiangen Biotech Co., Ltd.), laminar flow hood (Shanghai Jinghong Experimental Equipment Co., Ltd.), biochemical incubator (Beijing Liuyi Instrument).

2.2 Experimental Procedures

2.2.1 Observation of Luoshan Strain Fruiting Body Morphology

2.2.2 Genome Extraction

Firstly, the LS sample strain was thoroughly crushed in a grinder and set aside for later use. Then, following the steps of the fungal genome extraction kit, the specific operational procedures are as follows:

(1) Take a 15 ml centrifuge tube, add 200 μL of pretreatment solution and three glass beads, then add an appropriate amount of Ganoderma fruiting body sample, place it in the grinder, and grind thoroughly.

(2) Add 20 μL of Proteinase K solution, mix it thoroughly with the fungal fruiting body powder, incubate at 37°C for 30-60 min, then add 200 μL of lysis solution, manually shake gently to mix well, and keep at 70°C. Add 200 μL of anhydrous ethanol, mix well, briefly centrifuge to remove liquid drops from the tube

walls.

(3) Pass the obtained solution through an adsorption column, wash with wash solution once, and rinse solution twice. Then, leave the adsorption column at room temperature for 5-10 min to allow any residual rinse solution in the adsorbent to dry thoroughly.

(4) Transfer the adsorption column to a new centrifuge tube, suspend 50-100 μL of ultrapure water in the middle of the adsorption membrane, let it sit at room temperature for 5-10 min, briefly centrifuge in the centrifuge (12000 rpm, 2 min), and collect the resulting solution in the centrifuge tube as a template for later use.

2.2.3 Amplification and Determination of 18SrDNA-ITS Sequence

Using universal primers for the fungal ribosomal gene transcribed spacer region:

PCR forward primer ITS1: 5'-TCCGTAGGTGAACCTGCGG-3'.

PCR reverse primer ITS4: 5'-TCCTCCGCTTATTGATATGC-3'.

PCR amplification system and program are shown in Table 2-1.

Table 2.1 – **PCR reaction system**

Reaction system	Dosage (ul)	Amplification	Procedure cycle
Super Mix	15	96°C 5 min	
ForwordprimerITSI(10p) ReverseprimerITSI(10p)	1	96°C 20 s	
	1	56°C 20 s	35
template(ng/μL)	1	72°C 30 s	
ddH_2O	12	72°C 10 min	
Total	30	16°C forever	

2.2.4 Detection and Purification of ITS-PCR Products

(1) Three microliters of PCR products were subjected to agarose gel electrophoresis with a 1.0% agarose gel at 150V, 100mA for 20 minutes. The bands were observed during this process.

(2) Subsequently, the purified DNA products were analyzed. The purification of PCR products followed the standard operating procedure for magnetic bead purification. This method utilizes the principle of magnetic beads to adsorb or release charged substances. DNA is adsorbed in a high salt, low pH solution and released in a low salt, high pH solution, achieving the separation and purification of DNA products.

2.2.5 Sequence Determination

PCR amplification products were detected by 1.0% agarose gel electrophoresis and imaged and saved using a UVP gel imaging system. After recovery and purification of the PCR amplification products, sequencing was performed using the PCR primers as sequencing primers by Huada Genomics. The sequences were processed using BioEdit and Sequin software and submitted to GenBank [27].

2.2.6 Sequence Alignment

The obtained sequencing results were aligned using NCBI-BLAST to identify and download sequences with more than 95% similarity. The NCBI website link is: https://www.ncbi.nlm.nih.gov/.

2.2.7 Sequence Alignment and Phylogenetic Tree Construction

The obtained sequences of the strain were aligned with the sequences found in GenBank-BLAST. The homologous sequences were analyzed, and based on the top ten sequences, all of which were identified as Ganoderma lucidum Karst, it was preliminarily inferred that the LS sample strain belongs to the Ganoderma lucidum species. Furthermore, the obtained sequences were edited using BioEdit, and in conjunction with some reported ITS sequences of Ganoderma species in GenBank, multiple sequence alignments were performed using ClustalX, complemented with manual comparisons. Genetic analysis of the experimental strain was conducted using MEGA-X, and a phylogenetic tree was constructed based on the Kimura two-parameter model, utilizing the Neighbor-Joining (NJ) method to delete all gapped

positions or missing data in the alignment results. The reliability of the phylogenetic tree was tested using the Boot.Strap program with 1000 iterations, and other parameters were set to default values [27].

2.3 Experimental Results and Analysis

2.3.1 Analysis of LS Strain External Morphology

The external morphology of Ganoderma is shown in Figure 2.1. The cap of the fungus is semi-circular and fan-shaped, with a brown outer surface, and the stem is purplish-brown, smooth on the outside and exhibits a lacquered sheen when dried. The bottom of the cap is white.

Figure 2.1 – Ganoderma lucidum appearance shape map

2.3.2 Observation of fruiting body of LS strain by scanning electron microscope

The scanning electron microscope image of Ganoderma from Luoshan is shown in the figure. Upon observation under the electron microscope, the upper surface of the cap is smooth. The pores of the Luoshan Ganoderma are long and dense, a structure that facilitates the absorption of moisture and the transport of nutrients.

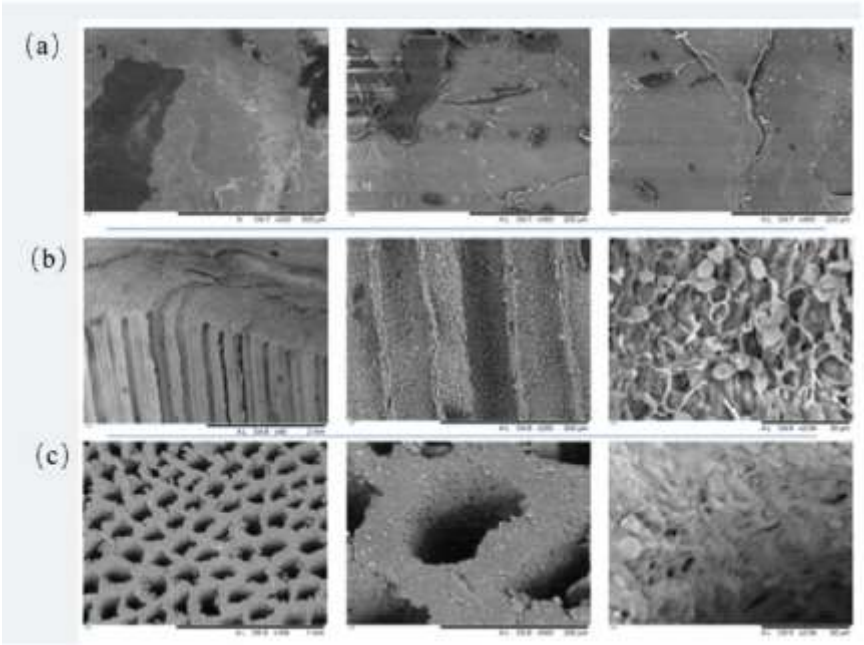

Figure 2.2 – Scanning electron microscope image of ganoderma lucidum cap

2.3.3 ITS Sequence PCR Results

The electrophoresis image of the PCR amplification using specific primers is shown in Figure 2-3. The PCR product fragment size of the test strain is between 500bp to 750bp. According to relevant literature, this range aligns with the typical length of the ITS region of fungal DNA.

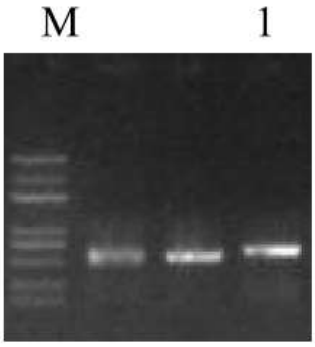

Figure 2.3 –Electrophoretic image

2.3.4 ITS final sequencing results

The sequence length of 635bp was finally obtained by sequencing, and the results were as follows.

CCGTAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTGACCGGGTTGTAGCTGGCCTTC
CGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTCAG
ATTGCGAGGCACGCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAA

ACTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATACAACTTTCAGCAA
CGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGA
GGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTGTGGTTTGTAGG
CTTGGACTTGGAGGCTTGTCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGG
TTCCTTGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTGGC
GAGCTTCTAACCGTCTTATAAGACAGCTTTATGACCTCTGACCTCAAATCAGGTAGGACT
ACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA.

2.3.5 Sequence Alignment and Analysis Results

The sequencing results were subjected to a BLAST sequence similarity search on the NCBI website. Suitable sequences were selected based on species classification for multiple sequence alignment. It was found that the sample "Luoshan Ganoderma" is most closely related to Ganoderma lucidum, as shown in Figure 3. By querying other Ganoderma base sequences from the GenBank database and conducting comparisons, a phylogenetic tree was constructed using MEGA-X software as shown in Figure 2-3.

2.4 Summary of This Chapter

The strain collected from Luoshan Scenic Area, upon observation of external morphology and scanning electron microscopy, was identified as belonging to the Ganoderma fungus. The total DNA of the fungus was extracted using a fungal total DNA extraction kit, and the ITS region sequence of 18SrDNA was obtained through PCR amplification. The obtained sequence was subjected to homology comparison analysis for molecular identification and species phylogenetic relationship determination, primarily through searches in the GenBank database and construction of a phylogenetic tree. The results indicated that "Luoshan Ganoderma" belongs to "Ganoderma lucidum".

CHAPTER 3
EXPERIMENTAL PART

3.1 Cultivation of Cultivated Ganoderma Mycelium

3.1.1 Experimental Materials:

A wild fungal fruiting body collected in 2020 from Luoshan Scenic Area in Yantai, Shandong.

3.1.2 Experimental Reagents:

experimental Instruments	Model	Manufacturer
Magnetic Stirrer	84-A	Shanghai Sile Instrument Co., Ltd
Vertical Electric Steam Sterilizer	LDZX-50KB	Shenan Medical Equipment Factory
Digital Constant Temperature Water Bath	HH-6	Guohua Electric Appliance Co., Ltd.
Electronic Analytical Balance	FA1204B	Aohaus (Shanghai) Instrument Co., Ltd.
Digital Benchtop Constant Temperature Shaker	HZQ-AStandard Model	Labwit Scientific GmbH (Germany)
Laminar Flow Hood	SW-CJ-1D	Suzhou Purification Co., Ltd.
Constant Temperature Blast Drying Oven	DHG-9053	Wujiang Innovation Oven Manufacturing Co., Ltd.
Constant Temperature Oscillating Incubator	LRH-70	Shanghai Jinghong Experimental Equipment Co., Ltd.
Water-jacketed Constant Temperature Incubator	JC-SHP-30	Shanghai Jinghong Experimental Equipment Co., Ltd.
UV-Visible Spectrophotometer	UV-5500PC	Shanghai Yuanxi Instrument Co., Ltd.

Glucose, yeast extract powder, Potato Dextrose Agar (PDA) medium, distilled water.

3.1.3 Experimental Instruments:

Preparation of the main solutions for culture media is as follows:

Basic solid culture medium formulation: PDA medium, 1g potassium dihydrogen phosphate, 0.5g magnesium sulfate, 2g peptone, 3g yeast extract powder, diluted with distilled water to 1L.

Liquid culture medium formulation: 20g glucose, 3g potato extract powder, 1g

potassium dihydrogen phosphate, 0.5g magnesium sulfate, 2g peptone, 3g yeast extract powder, diluted with distilled water to 1L.

3.2.1 Experimental Method

Isolation of the Fruiting Body and Reverse Tissue of collected Luoshan Ganoderma:

Scrape a small amount of white powder from under the cap of the Luoshan Ganoderma strain using a spatula, mix it with 1ml of sterile water, spread it on the basic solid fermentation medium, place it in a constant temperature incubator at 23 degrees, allow colonies to grow in the medium, scrape the colonies again until a single colony grows. Inoculate the mycelium onto the solid culture medium. Inoculate the obtained sample strain mycelium into the fermentation medium, with one group placed in a constant temperature incubator at 30 degrees for static cultivation, and another group placed in a shaker at 30 degrees and 200r/min for 8 days of cultivation, observing its morphology.

3.3 Identification Results of Cultivated Mycelium ITS

CAAAACTCGATAATGATCCTTCCGTAGGTGAACCTGCGGAAGGATCATTAT
CGAGTTTTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGC
TCATCCACTCTACACCTGTGCACTTACTGTGGGCTTCAGATTGCGAGG CAC
GCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACA AACT
CTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATAC AACTTT
CAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG AAATGC
GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT TGAACGC
ACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGT GTCATGAA
ATCTTCAACCTACAAGCTTTTGTGGTTTGTAGGCTTGGACTTGGAGGCTTG
TCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGG
ATCGGCTCTCGGTGTGATAACGTCTACGCCGCGACCGTGAAGCGTTTGGCG
AGCTTCTAACCGTCTTATAAGACAGCTTTATGACCTCTGACCTCAAATCAG
GTAGGACTACCCGCTGAACTTAAGCATATCA

CONCLUSIONS

Experimental Results

The Ganoderma mycelium solid culture plate, plate 1, was contaminated with fermentation, while the other three plates were not contaminated.

Observing the culture dishes, the colonies were evenly distributed, and the mycelium appeared as white, spreading outwards in a circular shape. The spores were brown, with a darker, deep brown color. The amount of mycelium varied on different culture media.

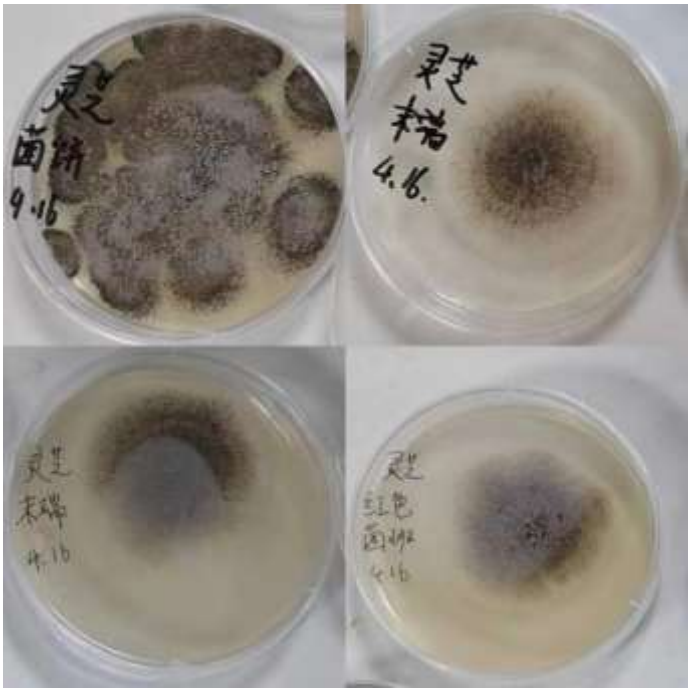

Figure 4.1 - Solid Culture Plate of Ganoderma Mycelium

In this study, through the research on wild Ganoderma and the comparative study of artificially cultivated Ganoderma, the external morphology of wild Ganoderma was determined through electron microscopy observation, and sequencing was performed. The obtained sequence was compared, resulting in the strain being identified as belonging to the *Ganoderma lucidum* species.

Subsequently, purified cultivation of Ganoderma was conducted, and solid culture and shaker culture were performed to obtain Ganoderma mycelium and mycelial balls. DNA extraction was carried out, followed by amplification of the ITS

sequence. Through sequence comparison, it was found that the DNA of the wild and cultivated Ganoderma mycelium was similar, both belonging to the Ganoderma lucidum species. It was concluded that the DNA sequence of the artificially cultivated Ganoderma did not undergo significant changes, maintaining consistency with the wild Ganoderma.

However, the analysis of metabolite accumulation revealed potential differences in the composition of metabolites between wild and cultivated Ganoderma, such as varying levels and proportions of polysaccharides, triterpenoids, and polyphenols. Cultivated Ganoderma exhibited an advantage in the accumulation of active components. In terms of medicinal activity evaluation, the extracts of wild and cultivated Ganoderma may differ in medicinal activities such as antioxidant, anti-inflammatory, and anticancer activities.

In terms of environmental adaptability, wild Ganoderma may demonstrate stronger adaptability under environmental stress, while cultivated Ganoderma may exhibit better stability and reproducibility under industrial production conditions. Based on the research results, this study can provide a scientific basis for the optimized cultivation and high-value utilization of Ganoderma, as well as serve as a reference for the application of different types of Ganoderma in the fields of medicine and healthcare.

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