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 fermentation of two strains of *Sophora japonica* mycelium**

First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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Kyiv 2024

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APPROVE
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«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Sun Xiaomeng

1. Thesis topic **Comparative study on the fermentation of two strains of *Sophora japonica* mycelium**
Scientific supervisor Ihor Hretskyi, Ph.D., As. prof

approved by the order of KNUTD "__"_____2024, №____

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

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

4. Date of issuance of the assignments_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Section 1 Literature review	From 06 April 2024 to 20 April 2024	
3	Section 2 Object, purpose, and methods of the research	From 21 April 2024 to 30 April 2024	
4	Section 3 Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 12 May 2024 to 24 May 2024	
7	Submission of qualification work to the supervisor for feedback (14 days before the defense)	From 24 May 2024 to 10 June 2024	
8	Submission of bachelor's thesis to the department for review (12 days before the defense)	13 June 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	15 June 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	17 June 2024	

I am familiar with the task:

Student _____ Sun XIAOMENG

Scientific supervisor _____ Ihor HRETSKYI

SUMMARY

Xiaomeng Sun. Comparative study on the fermentation of two strains of Sophora japonica mycelium. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2024.

The pure culture was obtained by isolation and purification from the bacteria picked from dead trees on the mountain. Solid fermentation culture was carried out at 28 ℃, 30 ℃ and 37 ℃, and the optimal growth temperature of the target strain was found out to be 30 ℃. Liquid fermentation culture was carried out on the mycelia of the bacteria, respectively, for static culture. The optimal growth data were obtained by shaker culture and shaker culture with Lactobacillus reuteri 56 added. The DNA genomes of two different strains of Pseudosophora Sophora, SJ-C strain and SJ-D strain, were amplified by PCR, and the amplified fragments were analyzed by gel electrophoresis. After purification, the amplified product was sequenced, and BLAST homology comparison was performed on the target fragment using ICGC database. The obtained sequence was analyzed using MEGA software, and a phylogenetic tree was constructed. The tree diagram was observed to compare and identify the sequence. The most closely related strain of SJ-C was Bjerkandera fumosa, and the SJ-C strain was identified as subsipihonia. The closest relative of this sequence of SJ-D strain was Ganoderma gibbosum, and SJ-D strain was identified as Ganoderma lucidum. The fermentation solution was sampled regularly, and the PH meter and hand-held refractometer were used to determine the change of acidity and reducing sugar content of the fermentation solution. The PH content of the measured solution decreased steadily with the increase of days and became weakly acidic. The reducing sugar content decreased slightly with the increase of days. The changes and effects of the relevant data of each stage of fermentation were analyzed and studied.

Keywords: Sophora pseudospora; Fermentation;Determination of pH ; reducing sugarsSophora pseudospora.

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INTRODUCTION

Fungi are a widely distributed group of low-growing plants in nature. They can be divided into the phyla of bacteria, slime molds, and fungi. In recent years, China has made significant progress in the development of edible fungi, ranking among the top in the world. There are numerous types of edible fungi, with 936 known species in China belonging to 41 families and 132 genera. Among them, basidiomycetes account for 94.4%, ascomycetes account for 5.6%, and there are approximately over 60 commercially cultivated species.

Trametes robiniophila Murr., commonly known as "Huai Ear," has medium to large fruiting bodies, corky texture, and no stalk. It mostly grows on the trunks of broad-leaved trees such as locust and sophora, with wild varieties appearing on dead trees, living trees, fallen trees, and stumps in summer and autumn. Its cap is semicircular, often overlapping like tiles, ranging in color from light brown to dark brown or white to gray-white or pale yellow, with blunt or sharp edges and a nearly smooth surface. The spores are colorless, smooth, oval to spherical, belonging to wood-rotting fungi, causing decay of the heartwood of trees. It is a white-rot fungus with the ability to degrade lignin.

Vanderbylia robiniophila, also known as "Huai Ear," is an extremely valuable medicinal macrofungus with a history of use in traditional Chinese medicine for 1600 years. It belongs to the phylum Basidiomycetes, class Agaricomycetes, order Polyporales, family Polyporaceae, and genus Vanderbylia. It is mainly distributed in the northeastern, eastern, and southeastern regions of China.

Medicinal fungi refer to a class of fungi that can treat diseases and have medicinal value, i.e., fungi that have health benefits for humans and can prevent, inhibit, or treat diseases. Some of these species not only have medical and health benefits but are also edible, known as medicinal and edible fungi, broadly referred to as medicinal fungi. Huai Ear is mainly used to treat diseases such as tonsillitis, pneumonia, and hepatitis, with broad prospects for medical applications.

Huai Ear was documented as "Huai Zha" in Li Shizhen's "Compendium of Materia Medica," described as having a pungent taste, bitter flavor, and neutral properties, with the efficacy of stopping diarrhea, bleeding, treating hemorrhoids, and relieving prolapse. Modern medical research has found that Huai Ear has biological activities such as antioxidant, anti-tumor, and enhancing immune function. Its anti-tumor mechanism mainly includes inhibiting angiogenesis, inducing apoptosis of cells, reversing drug resistance, and inhibiting the invasion and metastasis of tumor cells. Currently, Chinese medicine products such as Huai Ear ointment and Huai Ear fungal extracts are widely used in the adjuvant treatment of gastric, lung, and breast cancers.

The relevance of the topic is the research of domestic and abroad.In recent years, with the development of the green circular economy model, the traditional industry of edible and medicinal fungi has seen various degrees of improvement in terms of industry models, supply chains, and industrial benefits driven by biotechnology. This article reviews the application of biotechnology in the production and deep processing of edible and medicinal fungi, covering common varieties, commonly used culture media, basic process flows, key process control points, and application directions. This provides insights for the innovation and upgrading of the edible and medicinal fungi industry.

In China's current market, many commercial fungal fermentation technologies have been developed. For example, through liquid fermentation experiments with Trametes robiniophila, the optimal liquid shake flask fermentation medium was determined to consist of 0.4% peptone, 2% glucose, 0.15% magnesium sulfate, and 0.2% potassium dihydrogen phosphate. Cultivation conditions are crucial factors in liquid fermentation yield. Through experimental exploration, the optimal cultivation conditions were determined to be an initial pH of 6.5, 100 mL liquid volume in shake flasks (250 mL Erlenmeyer flasks), agitation speed of 140 rpm, fermentation temperature of 26°C, and fermentation time of 10 days. The optimal cultivation

conditions were summarized from experiments, providing technical basis for large-scale production.

In existing research, the majority focuses on strain cultivation studies. For example, through the cultivation characteristics of several polypore fungi and the pharmacognostic research of Ganoderma applanatum, excellent strains were determined by analyzing the growth rate of strains in culture media, the extension length of mycelium in cottonseed shell medium on the 20th day, contamination rate, and bioconversion rate. Sequencing showed a high degree of consistency in Ganoderma applanatum sequences, with no apparent variation in base sequences. By constructing molecular phylogenetic trees of Ganoderma applanatum and Fomitopsis (commonly known as Huai Ear), it was shown that the closest phylogenetic relationship was between Ganoderma applanatum and Fomitopsis. Therefore, the study of Ganoderma applanatum is considered significant, providing a basis for further selection of alternatives for Huai Ear medicinal materials.

Furthermore, the regulation of secondary metabolites is a hot topic in current liquid deep fermentation. The physiological mechanisms of medicinal fungi are complex. Through liquid fermentation technology, optimizing cultivation conditions can purposefully obtain targeted secondary metabolites, such as polysaccharides, triterpenoids, etc. Adequate sources of mycelium and targeted metabolites can ensure sufficient and high-quality medicinal sources. In the field of medicine, the deep development of wild medicinal fungi is widely applied. For example, the fermentation liquid of Poria cocos is an important material for the treatment of nephritis capsules. Besides traditional research areas, large-scale fungal liquid deep fermentation has expanded into the field of biotransformation. For example, large-scale fungi are used for the fermentation of traditional Chinese medicinal materials, utilizing the rich enzyme system of large-scale fungi to conduct liquid deep fermentation on some traditional medicinal materials, to produce new active substances through microbial metabolism, or to reduce the absorption of some difficult-to-absorb macromolecules through microbial metabolism.

Foreign investigations have discovered significant potential for Huai Ear in cancer treatment. For instance, in breast cancer treatment, its anticancer mechanism may be related to various biological activities such as inhibiting cell proliferation, metastasis, tumor angiogenesis, promoting cancer cell death, and regulating tumor-specific immunity. Increasing evidence suggests that Huai Ear may be effective in clinical treatment of breast cancer. Huai Ear is also widely used in the treatment of hepatocellular carcinoma (HCC). According to foreign references, in vitro experiments have been conducted to study the therapeutic effects of Huai Ear on non-small cell lung cancer. Using bioinformatics methods to predict potential targets of Huai Ear and verifying them through immunoblotting, molecular docking, kinase activity assays, and cellular thermal shift assays (CETSAs) further elucidate the targeting mechanism of Huai Ear. Finally, the in vivo efficacy was further verified. Huai Ear inhibits proliferation and promotes apoptosis of non-small cell lung cancer cells. From a bioinformatics perspective, epidermal growth factor receptor (EGFR) may be a target of Huai Ear. Western blotting results showed that Huai Ear attenuated the activation of EGFR and interacted with EGFR. In vivo, Huai Ear significantly inhibits tumor growth by attenuating the expression of p-EGFR. This study provides new insights into Huai Ear and demonstrates its potential as a therapeutic drug.

Extracts of Huai Ear components also play a significant role in medicine. For example, Huai Ear polysaccharides can alleviate acute cardiotoxicity induced by doxorubicin by regulating iron descent.

From a research method perspective, the research methods and technical routes of the above works and many papers have provided important inspiration for this article. Overall, Huai Ear has been widely used in anti-tumor therapy and postoperative recovery, with tremendous medical value. Although there are many health products on the market made from Huai Ear, there is a lack of scientifically effective quality control methods. Its production scale and technological content have not yet met market demand. Further research and development of Huai Ear products are needed.

The purpose of the study is the Huai Ear fungal extracts are produced using solid-state fermentation, while mycelium is produced using liquid culture methods. Liquid fermentation involves preparing a liquid medium containing the necessary nutrients for mycelial growth, starting from test tube inoculants, gradually scaling up the culture volume, and then fermenting in shake flasks or fermenters to achieve large-scale cultivation of mycelial aggregates. Shake flask cultivation is a commonly used aerobic cultivation method in laboratories, where liquid culture medium in Erlenmeyer flasks is placed on a shaker for agitation, meeting the oxygen demand for microbial growth, reproduction, and the production of numerous metabolic products. It is a common method for exploring process conditions in the laboratory. For instance, liquid fermentation of Ganoderma lucidum can produce a large quantity of mycelium, and the content and types of bioactive substances produced are essentially consistent with those of naturally harvested Ganoderma fruiting bodies, with some important active substance levels even higher than those found in the fruiting bodies.

The objectives of the study about that there is a dearth of comparative research on various locust fungi fermentation methods. Wild resources are becoming increasingly scarce, while artificial cultivation cycles are lengthy and costly. Consequently, the overall utilization value of locust fungi remains low, impeding their development. Utilizing deep fermentation culture can expedite growth and reproduction, allowing for rapid extraction of mycelium and resultant metabolites. This facilitates extensive extraction of active components and enables thorough product analysis and identification.

Given these circumstances, this study primarily aims to optimize the formulation of mycelium fermentation components and enhance fermentation processes and production capacity. By employing diverse spore purification techniques, solid and liquid cultures, as well as seed preservation methods, we aim to systematically evaluate mycelium components through fermentation and subsequent analysis. Ultimately, this research endeavors to furnish a scientific foundation for the future development and utilization of locust fungi.

The object of the study Comparative study on the fermentation of two strains of Sophora japonica mycelium

The subject of the study Comparative study on the fermentation of two strains of Sophora japonica mycelium

Research methods:

This experiment involved the isolation and purification of five different strains of *Trametes robiniophila* (A/B/C/D/E) through multiple single-colony picks. Each strain was cultured both on solid and liquid media, with three sets of control experiments: carbon source, nitrogen source, and a blank control. The effects of different fermentation media on different strains were analyzed under various fermentation temperatures, initial pH values of the culture medium, and agitation speeds, aiming to identify the optimal culture conditions in different cultivation environments. Preservation methods for the strains were studied. The mycelial composition was examined, and genomic DNA was extracted for PCR amplification. The resulting products were then tested, identified, and subjected to ITC sequence determination. Sequencing results were compared against Genbank using BLAST to construct a phylogenetic tree with sequences showing over 95% similarity. Titration was employed to determine the acidity of the fermentation broth, while the DNS colorimetric method was used to determine the content of reducing sugars to explore unknown substances produced and consumed during the fermentation process.

1.Screen and purify single colonies;

2.Prepare PDA liquid medium, LB solid medium;

3.Culture colonies at different temperatures, observe and record colony growth, and select the optimal temperature for colony growth;

4.Solid-state fermentation to identify the most suitable components of the medium (carbon source, nitrogen source);

5.Liquid fermentation culture: transfer activated Huai ear fungus strains to 200 mL PDA liquid medium, culture at 28°C, 160 rpm on a shaker bed to obtain fermentation broth;

6.Effect of shaker speed on liquid fermentation of Huai ear fungus;

7.Preservation of strains;

8.Taxonomic identification: morphological identification, ITS sequence analysis;

9.Titration method to measure the acidity of the fermentation broth. Daily, take the fermentation broth and filter it through four layers of gauze. According to the principle of acid-base neutralization, titrate with alkali solution, using phenolphthalein as an indicator to determine the end point of titration. Calculate the total acid content based on the consumption of alkali solution, with three parallel experiments and a blank control group;

10.Use DNS colorimetric method to determine the content of reducing sugars in the fermentation broth. Daily, take the fermentation broth and filter it through four layers of gauze. Based on the absorbance-glucose concentration standard curve at 540nm wavelength, calculate the concentration of reducing sugars in the fermentation broth and determine the content of reducing sugars.

The scientific novelty:

Currently, there is a lack of comparative research on the fermentation of different Cordyceps sinensis mycelia. Due to the gradual depletion of wild resources and the relatively long cultivation period and high cost of artificial cultivation, the utilization value of Cordyceps sinensis has been affected, thereby hindering its development. To overcome this issue, this study adopts deep fermentation cultivation technology to promote the growth and reproduction of Cordyceps sinensis mycelia through solid-state and liquid-state cultivation. This method enables the rapid acquisition of mycelia and their metabolites, facilitating the effective extraction of active ingredients for analysis and identification. Based on the above considerations, this study aims to optimize the composition of mycelial fermentation, improve fermentation processes, and increase production capacity. Specific operations include spore isolation and purification, solid-state cultivation, liquid-state cultivation, seed preservation, and fermentation of different Cordyceps sinensis mycelia, followed by

inspection and determination of mycelial components, and comparative analysis of the results, in order to provide a scientific basis for subsequent development and utilization.

The practical significance of the results obtained is that from a methodological perspective, the research methods and technical routes outlined in the aforementioned works and numerous papers have provided significant inspiration for this study. Generally speaking, Huai ear fungus has been widely applied in various health maintenance, therapeutic, and preventive efforts across diverse life forms, such as in anti-tumor treatments, postoperative recovery from tumors, anti-inflammatory purposes, and the reduction of blood pressure and lipids, demonstrating substantial medical value.

CHAPTER 1
LITERATURE REVIEW

1.1 *Sophora japonica* mycelium

Bacteria are a low plant that is widely distributed in nature. They can be divided into bacteria, myxobacteria, and fungi. Nowadays, China has made rapid progress in the development, utilization and development of edible fungi, and also occupies a place in the international edible fungus industry [1]. There are many kinds of edible fungi. There are 936 known species in China, respectively belonging to 41 families and 132 genera, most of which belong to basidiomycota 94.4% and ascomycote 5.6%. On the market, there are about 60 kinds of [2-4] for commercial cultivation.

Sophora japonica mycelium, also known as locust ear, locust fungus, etc., fruiting body sessile, usually medium to large, wood plug, is a relatively common wild edible fungus, belongs to the porous fungus, porous fungus genus. Huai fungus with its unique appearance and rich medicinal effect and by the majority of consumers love. At present, it is distributed in most areas of China, mainly in the northeast, East and southeast regions of China [5]. They mostly grow on the trunks of broad-leaved trees such as locust, locust and green sandalwood, while wild locust grows in dead trees, living trees, inverted trees and stumps in autumn. Its fungus cap is semicircular, often covered with tile shape, light brown to dark brown or white to grayish white or light yellow, blunt or sharp edge, the surface is nearly smooth. A colorless, smooth, oval to spherical spore, a wood rot fungus, is a white rot fungus with the ability to degrade lignin [6]. It contains a variety of essential amino acids, vitamins, trace elements, especially it contains more crude protein, crude fat and polysaccharide and other nutrients, and it is a kind of rare edible fungus with high economic value and medicinal value.

1.2 The function of Sophora japonica mycelium

1.2.1 Antineoplastic

Sophora japonica mycelium and its fruiting body in the field of anti-tumor have shown its potential therapeutic value. Locin, the active ingredient of locust, can effectively inhibit the growth of tumor cells, enhance the body immunity, and inhibit tumor cell metastasis. It has been widely used in the adjuvant treatment of a variety of malignant tumors, including liver cancer, lung cancer, leukemia, malignant lymphoma, breast cancer, ovarian cancer and gastric cancer [7-8]. Its anti-tumor mechanism mainly involves [8] in inhibiting the growth and proliferation of tumor cells, inhibiting angiogenesis, inducing the apoptosis of tumor cells, inhibiting the invasion and metastasis of tumor cells, regulating the expression of various proto-oncogenes and tumor suppressor genes, improving the immune ability of the body, and promoting the reversal of drug resistance in drug-resistant tumor strains.

The chemical components of locust ears mainly include polysaccharides, steroids and alkaloids [7]. The crude polysaccharides extracted from the fruiting bodies of locust ears can play a tumor inhibitory role by increasing macrophages, thus promoting the proliferation of lymphocytes and the activity of natural killer cells. Together, these components act on tumor cells to exert their anti-tumor effects. For example, locust alkali is an important component in the locust ear, in vitro experiments found that it can concentration related inhibit the proliferation of a variety of tumor cells, including colorectal cancer, gastric cancer, pancreatic cancer, liver cancer, esophageal cancer and other [9].

Huai ear not only showed good anti-tumor activity in vitro, but also was validated in clinical applications. Huai ear granules have been widely used in the treatment of a variety of malignant tumors in clinical practice, all of which have certain curative effect, including osteosarcoma, leukemia, malignant lymphoma, breast cancer, lung cancer, rectal cancer, liver cancer, pancreatic cancer, gastric cancer, esophageal cancer and other [10]. This indicates the broad applicability and effectiveness of locust ear and its extracts in antitumor.

Studies in the antitumor immune response to the locust ear show that it improves immune function by regulating innate and adaptive immunity and exerts indirect antitumor effects [11]. This mechanism of action further illustrates the potential of the locust ear in tumor therapy.

The study of locust fungus and its fruiting body in the field of anti-tumor has shown its remarkable research and application value. Its diverse chemical components and complex antitumor mechanisms have great prospects for development and utilization in the area of antitumor adjuvant therapy. In future studies, the specific mechanism of action of locust ear and how to optimize its use strategy to improve its efficacy and safety in clinical treatment.

1.2.2 Antiinflammatory

Sophora japonica mycelium can effectively inhibit the production of inflammatory mediators, reduce inflammation, improve the local tissue microcirculation, and have a certain therapeutic effect on the inflammatory symptoms such as local red, swelling, heat and pain. For example, we found the change of composition content and anti-inflammatory activity in the process of "locust ear-root fermentation", which provided a new idea and direction for the processing of traditional Chinese medicine and the research and development of new drugs. After the bidirectional fermentation of locust ear plate and blue root, the content of polysaccharide and inosine with anti-inflammatory and anti-tumor effects in the fermentation system gradually increases, and the fermentation fungus matter also has good anti-inflammatory activity. Therefore, we know that the bidirectional fermentation technology, as a new type of TCM processing technology, can not only enhance the efficacy, but also expand the scope of medicinal use, which has a broad development prospect in clinical medicine and application [12]. At the same time, it also proves that the new idea of solid bidirectional fermentation has some rationality and practicability, which is expected to provide scientific basis for the medical community.

1.2.3 Anti-thrombotic

The antithrombotic effect of Sophora japonica mycelium is mainly reflected in the fact that locust bacteria can effectively remove platelets and red blood cells in the blood and prevent blood coagulation. First, Sophora japonica and its extracts showed some effects in achieving hemostasis and improving blood rheology. Sophora charcoal, fried Sophora, Sophora and its extracts tannin, quercetin, rutin can reduce capillary permeability. In animal experiments, using mice and rats as models, we observed that Sophora japonica extract significantly shortened the length of bleeding and coagulation in mice, and effectively reduced the activation time of prothrombin in rat plasma. In addition, Sophora extract also increased fibrinogen content and significantly inhibited the aggregation rate of rat platelets. These findings together support the important conclusion that P. japonica and its extracts show a potential positive effect in preventing thrombosis.

The experience of Chief physician Sun Wenliang also supports the effectiveness of Sophora japonica in the treatment of thrombophlebitis. His reuse of Sophora japonica with cold blood hemostasis and heat clearing and fire in the treatment of thrombophlebitis often achieved good results, which further proves the potential of Sophora japonica and its related components in antithrombosis [13].

Although the evidence directly on antithrombosis is insufficient, the results of Sophora and its extracts may suggest that locust or its related components may have antithrombotic effects. These findings provide indirect support for the antithrombotic role of A. spp.

1.2.4 Reduce blood press

It can effectively improve the heart function and reduce the blood pressure. Sophora japonica and its related components have some research basis and clinical application value in lowering blood pressure. For example, in medical practice, compound Sophora pressure is widely used in the treatment of insulin resistant hypertension, and its effect is remarkable. Moreover, the drug also showed positive

regulatory effects on blood lipid levels and glucose tolerance, and showed regulatory effects on blood lipid and glucose tolerance, providing an effective treatment option for hypertension and its associated metabolic abnormalities. This finding not only expands the application scope of Sophora drugs, but also provides a new strategy for the treatment of hypertension and metabolic diseases. In addition, the combination of baicalensis-sopjaponica is considered to effectively treat hypertension and its kidney injury, its mechanism of action may involve improving the intestinal flora disorder, repair intestinal barrier function, reduce sulfate and inhibit the renal oxidative stress response, increase flora metabolites SCFAs, regulate SCFAs related receptor levels and reduce inflammation response [14].

The direct evidence on locust bacteria has not been explicitly mentioned, but it can be inferred that locust flowers and its related components do have potential applications in lowering blood pressure. The compound preparation of Sophora has shown some effect in the treatment of insulin resistant hypertension, which provides a scientific basis for the development of new blood pressure therapy.

1.3 Overview of the identification techniques for Sophora japonica mycelium

The identification techniques of locust bacteria mainly involve morphological analysis, molecular taxonomy and chemical analysis. Based on the morphological characteristics and microstructure of fruiting bodies is the main basis for the classification and identification of traditional edible fungi. However, even for different strains, some have similar morphological and microscopic characteristics, so the identification and classification of strains by morphological characteristics alone often produces some bias [15]. Therefore, correct naming and classification of bacteria using molecular taxonomy became a key approach to solve this problem. It further shows that the method of molecular taxonomy can provide scientific theoretical basis for the further research and utilization of thrombobacteria.

Secondly, from the perspective of chemical analysis, the study of the exopolysaccharides shows that through infrared spectroscopy, gas phase mass spectrometry (GC/MS) analysis and thermal weight analysis, the exopolysaccharides produced by the tree have clear structural characteristics, and shows significant functional effects in the production and application of tobacco flakes. Through the intensive study of these polysaccharides, we can better understand the mechanism of action in tobacco flakes and provide the scientific basis for the improvement of tobacco products. These chemical analysis methods not only revealed the polysaccharide containing β-glycosidic bond, glucose, ribose, arabinose and mannose components, also found in the processing temperature should not exceed 195℃, and added to tobacco slices can significantly improve the quality of cigarette [16].

To sum up, the identification technology of bacteria covers the molecular taxonomy, using molecular classification of bacteria correct name and classification, and with the help of advanced chemical analysis means such as infrared spectroscopy, gas chromatography-mass spectrometry (GC/MS) technology and thermal weight analysis, further study of the structural characteristics of polysaccharide and its effectiveness in practical application. The application of these techniques helps to solve the confusion between the bacteria, and also provides a scientific basis for the application of locust bacteria and its exopolysaccharides.

In recent years, with the development of green circular economy model, the traditional industry of food, medicinal and fungi has improved the industrial chain, industrial model and industrial benefits. The application of biological fermentation technology in the field of edible and medicinal fungi is evaluated from the aspects of common varieties, common culture media, basic process flow, process control key points and application direction, which provides ideas for the innovation, development and upgrading of food and medicinal fungi industry [17].

Today's Chinese market has developed many commercial strain fermentation technology. In existing research, to strain culture research accounted for the vast majority, the regulation of secondary metabolites is the hot spot of liquid deep fermentation, in the field of medicine, the physiological mechanism of medicinal bacteria is very complex, through the liquid fermentation technology, optimize the culture process conditions, purposeful target secondary metabolic active substances, such as polysaccharide, three blind substances, etc. At the same time, the acquisition of a large number of mycelium and target metabolites can also ensure sufficient drug sources and the quality of drug sources, so the deep development of wild medicinal bacteria can be widely used. Currently large fungal liquid deep fermentation in addition to the traditional research category, also expanded to the field of biological transformation, such as large fungal fermentation of Chinese herbal medicine, using large fungal rich enzymes, for some traditional medicine liquid deep fermentation, see can use the metabolism of microorganisms to produce new active substances, or the use of microbial metabolism and reduce the difficult to be directly absorption of large molecules [18].

Foreign investigations and studies have found that locust ear has great potential in cancer treatment. For example, in breast cancer treatment, its anticancer mechanism may be related to a variety of biological activities, such as inhibition of cell metastasis, proliferation, tumor vascular generation, promotion of cancer cell death, regulating tumor cell-specific immunity and so on. Increasing evidence suggests that the locust ear may be effective in [19](Luo F K 2024) in the clinical treatment of breast cancer. According to foreign references, in vitro experiments are used to study the therapeutic effect of locust ear on non-small cell lung cancer. Potential locust ear targets were predicted using bioinformatics methods and validated by immunoblotting. The mechanism [20] of locust ear targeting was further clarified by molecular docking, kinase activity measurement, and cell heat shift assay (CETSAs).

Conclusions to chapter 1

1.Enhance human immunity

Through the above literature reading, it is learned that it has great potential medicinal value, and its related components or products have anti-cancer, anti-inflammation, lowering blood lipid and blood pressure, which fully reflects that it can effectively improve human immunity, regulate the immune system, and enhance the body's ability to resist disease resistance. Locsinensis and its related components enhance human immunity through different mechanisms, including direct action on the immune system, modulation of the balance of gut microbial community, and applications as immune adjuvants and enhancers. These research results provide a scientific basis for the application of locust in enhancing human immunity, and attract a large number of scholars to study and analyze it, and play a role and value in ensuring human health.

2.Purpose of research

At present, there are few comparative studies on the fermentation of different locust fungi, the wild resources are gradually scarce, the cycle of artificial cultivation is relatively long and the cost is high, resulting in the low utilization value of locust fungi and hindering the development of locust fungi. Using deep fermentation culture to ensure the growth and reproduction, the mycelium and the resulting metabolites can be obtained in a short time, and the active components can be extracted to a large extent, and the products can be analyzed and identified.

In view of this, this study is mainly to optimize the formula of mycelium fermentation components, improve fermentation process and production capacity, through different spore purification, solid culture, liquid culture, seed preservation, fermentation, test the components of mycelium measurement and analysis results, in order to provide scientific basis for later development and utilization.

3.Research meaning

In China, the development of traditional Chinese medicine can be traced back to thousands of years ago. It is an important part of traditional Chinese medicine, such

as the Huangdi Neijing, Qianjin Fang, Compendium of Materia Medica and other medical works. With the development of society, the influence and status of TCM in China have been increasing, and gradually spread around the world, and its therapeutic function has been fully confirmed in the COVID-19 outbreak in recent years. Therefore, the research and development of the medicinal value of locust bacteria provide ideas for the innovation and development of new traditional Chinese medicine.

From the perspective of research methods, the research methods and research technical routes of the above works and many papers all give important inspiration to this paper. In general, locust has been widely used in the health care, treatment and prevention of various life bodies, such as anti-tumor treatment, postoperative tumor recovery, anti-inflammation, blood pressure and blood lipid reduction, etc., which has great medical value. At present, there are a large number of locust extracts on the market as medicinal and edible commodities, but due to the lack of scientific and efficient quality control means, the production scale and technical level can not meet the needs of the market, so the research and development of locust products need to be further deepened.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE STUDY

2.1 Design philosophy

It is produced by solid fermentation and mycelium by liquid culture. Liquid fermentation is a technique of making the most necessary nutrients for mycelium growth of mycelium into a liquid culture medium, starting from the test tube strain, increasing the culture step by step, and then conducting shake flask fermentation or fermentation by fermentor to expand the cultivation of mycelium ball. Shake flask culture is a common method of ventilation culture in the laboratory. Put the triangular bottle with liquid culture on the shaking aker to meet the needs of microorganisms and produce many metabolites for oxygen, it is a common method to explore the process conditions in the laboratory.

2.2 ITS sequential analysis

As a molecular biology technique, ITS sequence analysis has shown its unique value in the field of fungal identification. The ITS represents the endogenous transcriptional spacer region (Internally Transcribed Spacer), which is located between the fungal 18S,5.8S and 28S rRNA genes, including two regions, ITS 1 and ITS 2. The short ITS sequences are about 350bp for ITS 1 and 400bp for ITS 2, which makes them easy to analyze and compare and are therefore widely used for phylogenetic analysis of different species of fungi.

In ribosomal DNA (rDNA), the ITS represents the inner transcribed spacer, which does not bind to the mature ribosome and is therefore subject to less selective pressure. Because of this scenario, ITS sequences evolve relatively rapidly. This sequence is highly homologous within species and has varying degrees of variation among different species. This widespread sequence polymorphism is common in most eukaryotes; moderate length provides sufficient information; the ITS region is moderately repetitive in the nuclear genome and its repeat units are highly similar or

identical, so PCR products can be subjected for sequence analysis. These features make ITS sequences ideal molecular markers for interspecies identification, widely used in molecular taxonomy, genetic diversity and strain identification of fungi. Therefore, the construction of ITS sequences combined with phylogenetic trees has extensive applications in fungal taxonomy and strain identification [21, 22].

2.3 Agarose gel electrophoresis

Agarose gel electrophoresis is an effective method for isolating large fragments of DNA and can be used to extract macromolecular DNA. With the electric field, biological macromolecules such as DNA and RNA can move in the agarose gels. Since DNA molecules produce different migration rates according to their molecular weight size after agarose gel electrophoresis, agarose gel electrophoresis is also often used for the detection of PCR products. Agarose gel electrophoresis technology has become an extremely important analytical method in modern times, electrophoresis technology is often used in nucleic acid analysis, so electrophoresis technology has been widely used in various fields, has a pivotal role in molecular biology and biochemistry [23].

2.4 Product analysis

The acidity of fermentation broth is determined by acid-base titration, PH meter, automatic potential titration.

There are many methods to determine reducing sugars, including redox titration, DNA colorimeand hand-held refraction instruments. The detection of reduced sugars in locust fermentation broth is usually performed by the 3,5-dinitrosalicylic acid (DNS) method. Since the liquid color is proportional to the reduced sugar content in a certain concentration range, the linear relationship between the glucose concentration and the absorbance can be obtained by measuring the absorbance of the solution at the wavelength of 540nm. The detection wavelength was set to 540nm, the boiling water bath time was 8min, and the color rendering amount of the agent was 2.5 mL,

cooled with running water, and the reducing sugar in the fermentation liquid was allowed for 20min. With the best conditions selected by the above steps, the [24] of the glucose standard curve was drawn by DNS color development.

Handheld refrreader is an instrument to determine the physical constants such as liquid concentration, refractive index, double folding rate and optical property. It is composed of eyepiece, prism mirror, high refractive index prism, lens and ruler. Light travels at different speeds in different media, so photorefraction occurs when light moves from one transparent medium to another. The refractive rate is the ratio of the speed of light in the air to that in other matter. The handheld light reader can be used to determine the refracting index of the liquid, the solute content in the solution, the sugar content of the wine, the water content in the high sugar solution, the salinity of the seawater and the specific gravity of the battery electrolyte. It is widely used in petrochemical industry, light industry food, medicine and health and environmental protection and greening.

Conclusions to chapter 2

1.Fermentation Methods and ITS Sequence Analysis:

Solid-state fermentation produces Huai ear fungus, while liquid culture produces mycelium.

Liquid fermentation involves the preparation of liquid culture medium, starting from tube seed cultures, gradually scaling up the culture, and conducting shake flask or fermenter fermentation to expand the cultivation of mycelia.

ITS sequence analysis is a molecular biology technique used for fungal identification. ITS stands for Internal Transcribed Spacer, including ITS1 and ITS2 regions. They are of moderate length, facilitating analysis and comparison. ITS sequences exhibit high homogeneity within species and variability between different species, thus widely applied in fungal taxonomy and strain identification.

2.Agarose Gel Electrophoresis Technique:

Agarose gel electrophoresis is an effective method for separating and extracting large molecular DNA.

DNA molecules migrate at different speeds in agarose gel based on their molecular weights, commonly used for PCR product detection.

3.Analytical Methods:

Acidity determination methods include acid-base titration, pH meter method, automatic potential titration, etc.

Methods for determining reducing sugars include oxidation-reduction titration, DNA colorimetric assay, and handheld refractometer. The detection of reducing sugar content in Huai fungus fermentation broth typically employs the 3,5-dinitrosalicylic acid (DNS) method.

Handheld refractometer is utilized for determining liquid concentration, refractive index, and other physical constants, applied in fields such as petrochemicals, light industry food, medical hygiene, and environmental greening.

CHAPTER 3
EXPERIMENTAL PART

3.1 Experimental materials

Experimental raw materials: locust locust fungus picked on the mountain and on the campus, as shown in the figure below:

Figure 3.1 - Raw materials of locust

3.2 Experimental reagents

Table 3.1 - **for experimental reagents**

reagent	vender
Potato immersion powder	Sinopharm Group Chemical Reagent Co., LTD
peptone	Beijing Aboxing Biotechnology Co., LTD
B vitamin	Aladdin Reagent (Shanghai) Co., Ltd

Genomic DNA extraction kit	Tiangen Company
5×TBE buffer solution	BBI Life Science company
GelRed dyestuff	Biotium company
SmaIenzyme	NEB company
agarose	Sun horse

3.3 laboratory apparatus

Table 3.2 - **for experimental instrumentation**

instrument	vender
Vertical electric-thermal pressure steam sterilization cooker	Shanghai Shen'an Medical Equipment Factory
Water temperature incubator	Shanghai Jinghong Experimental Equipment Co., LTD
superclean bench	Shanghai Zhicheng Analytical Instrument Manufacturing Co., LTD
Digital constant temperature water bath	Guohua Electric Appliance Co., Ltd
BSM-220.4 One in ten thousand is an electronic balance	Shanghai Zhuojing Electronic Technology Co., LTD
84-1A magnetic stirrer	Shanghai Sile Instrument Factory
centrifuge	Jinan Hanlin Equipment Co., LTD
Digital display desktop constant temperature shaker oscillatorVeriti PCR	Germany Labwit Scientific company
	U.S.A. AB company
Basic electrophoresis apparatus trophoresis	U.S.A. Bio-Rad company

CHEF MAPPER Pulsed-field gel electrophoresis apparatus	U.S.A. Bio-Rad company
Gel DocXR+ Gel imaging systemPH meter	U.S.A. Bio-Rad company
Hand-held dimmer	Mettler-Toldo company
	Shanghai Optical Factory

3.4 Experimental strains

3.4.1 Acquisition of the experimental bacterial species

The experimental strains were obtained using tissue isolation and obtained pure cultures after multiple purification on PDA medium.

3.4.2 The configuration of the PDA solid medium

(1) Configuration of culture medium: accurately weigh the medicine required by the culture medium, then stir and dissolve it with a magnetic mixer, measure pH with a pH meter, adjust it to 7.4, finally divide it into a triangle bottle, and put it into an autoclaved steam sterilizer for sterilization.

(2) Inverted plate: when the medium is cooled to not hot (about 45℃ ~50℃), conduct experimental operation in the ultra-clean table to make 3 groups of PDA solid medium.

(3) The content of the poured culture medium is about half of the capacity of the plate. After the culture medium is solidified, the poured plate will be inverted and stored in time.

The components of PDA solid medium (per liter) were 15g agar powder, 10g trypsin, 5g yeast immersion powder, and 10g sodium chloride. Sterilization condition: 20min at 121℃.

3.4.3 The position of LB liquid medium

600 mL of LB liquid medium was prepared with distilled water, then the pH of the adjusted medium was measured using a pH meter to be 7.4, and it was evenly split into three triangular bottles.

LB liquid medium (per liter) contains 5g yeast immersion powder, 10g trypsin, and 10g sodium chloride. Sterilization condition: 20min at 121℃. PDA liquid medium: add 20g glucose, 3g potato powder, vitamin 0.01g, 1000 mL of distilled water to make liquid medium, fully mixed and divided into a triangular bottle, wrapped with filter paper film and newspaper, in a high temperature autopressure cooker 121℃, 20min.

3.5 Fermentation and culture of locust bacteria

3.5.1 culture solid

The configured solid medium was inoculated, purified and cultured, and the cultured strains were marked as A / B and dated for differentiation.

3.5.2 Temperature culture optimization

The strains were connected into PDA solid medium and incubated at four different temperatures: 28℃, 30℃ and 37℃ for 2-3 day to observe the growth of the strains at different temperatures. Observe the growth of mycelium or steamed buns on the plate, and take photos to record it.

3.5.3 liquid culture

(1) In the ultra-clean test table, prepare LB liquid culture medium, purified petri dish, the locust cake cake, the end of the locust cake (no hyphae), and sterilized distilled water.

(2) flush the surface of the spores with distilled water two to three times, scrape a few surface spores with the inoculation ring, connect them to the three conical

liquid culture medium, slightly swing the needle in the liquid culture medium; the three conical flasks are labeled A / B / C.

(3) Put conical bottle A into the incubator, B into 30.5℃ 200r into the combined shaker, and C into No.56 into the combined shaker 30.5℃ 200r into the shaker.

3.6 Measurement and observation indicators

3.6.1 Measurements of acid alkalinity

In this experiment, the pH was measured using a PH meter, and the liquid locust plug added with L. reuteri 56 was measured with 1000ul of a pipette gun 2-3 times in a small test tube.

As illustrated in following figure:

Figure 3.2 - Measurement of the pH

3.6.2 Measurements of reduced sugars

The method for measuring reducing sugars used in this experiment is a handheld refractor used as follows:

1. When using the hand-held fold-reader, first hold the rubber sleeve in the middle section of the instrument with the left hand to prevent the influence of body temperature on the measurement accuracy, and then use the right hand to adjust the eyepiece.

2. Gently open the light intake board and wipe the refractive prism clean with a cleaning cloth.

3. Use a pipette to take out several drops of culture fluid from the test tube and drop them on the refractive prism. Gently close the light plate to avoid generating bubbles and ensure that the solution is evenly distributed on the surface of the prism. Point the light inlet plate of the instrument to the light source, observe the light-shade boundary through the eyepiece, and record the reading [25].

Note: If the boundary between light and shade is not clear, you can adjust it with the right hand, and then rotate the zero screw, put the boundary between light and shade at zero, and then observe and record.

As illustrated in following figure:

Figure 3.3 - Measurement of reduced sugars

3.7 Molecular identification of P. sinensis

3.7.1 Genomic DNA extraction of the bacterial strains

1. First, the picked locust plug was ground into a powder, and then 400 μ l of Buffer A1 and 4 μ l of RNase A (10 mg/ml) were added to it, and fully mixed using a vortex oscillator to promote lysis. For high polysaccharide or high polyphenol samples, 2% PVP-40 or 0.2% β -mercaptoethanol were added to Buffer A1, or to both for optimal treatment.

2. The sample was heated at 65℃ for 10 minutes, during which the centrifuge tube was repeatedly reversed 2 to 3 times to ensure that the sample was evenly mixed together.

3. Heat the sample in a 65℃ water bath for 10 minutes, during which you can repeatedly reverse the centrifuge tube for 2 to 3 times to ensure that the sample is mixed evenly.

4. Follow the volume of the supernatant, add Buffer A3 equivalent to 1.5 times the volume (confirmed after adding absolute ethanol), and blow immediately to mix. Note that the addition of Buffer A3 may form a flocculent precipitate, after mixing to continue the next step.

5. Next, the mixture (including any precipitate) was transferred to the FastPure gDNA Columns IV already placed in the collection tube, centrifuged at 12,000 rpm for 30 to 60 seconds, and the filtrate was discarded.

6. Add 600 μ l of Buffer AW (confirmed to add absolute ethanol) to the column, centrifuge again at 12,000 rpm for 30 seconds and discard the filtrate.

7. Repeat the centrifugation steps as described above.

8. Prepare a new 1.5-ml centrifuge tube and place the adsorption column therein. Add 50 to 100 μ l of Elution Buffer preheated to 65 to 70℃ to the adsorption column center, leave at room temperature for 3 to 5 minutes, then centrifuge at 12,000 rpm (13,400 g) for 1 minute. Note that the elution volume should be at least 50 μ l to prevent elution efficiency decline; elution can be repeated with a new Elution Buffer to increase yield, although this may reduce concentration; if maximum yield is pursued, reuse the first elution is recommended.

9 Finally, the DNA from the adsorbed column was discarded and stored at-20℃.

3.7.2 Amplification and determination of the 18 S rDNA-RTS sequence

The amplification experiments mentioned in the article used the following universal primers for the transcribed spacer region of the fungal ribosomal genes, specifically listed below.

Table 3.3 **- for primer information**

Primer name	Primer sequences	Fragment size
ITS1	TCCGTAGGTGAACCTGCGG	400~800 bp
ITS4	TCCTCCGCTTATTGATATGC	

Table 3.4 **- PCR amplification reaction system and conditions**

reaction system	volume/ul	Amplification program		circulate
Super Mix	15	96 ℃	5 min	35
Primer F（10p）	1	96 ℃	20 s	
Primer R（10p）	1	56 ℃	20 s	
mould（ng/μL）	1	72 ℃	30 s	
ddH2O	12	72 ℃ 10min		
total	30			

3.7.3 The PCR products were detected and purified

First, 3 μ L of the PCR product was subjected to 1.0% agarose gel electrophoresis to detect the presence of specific bands. Subsequently, the PCR products were purified according to the magnetic bead purification technique. The basic principle of magnetic bead purification is to use magnetic beads to adsorb DNA in high salt low pH environment and release DNA in low salt high pH environment, so as to realize the separation and purification of DNA. BGI was responsible for

completing the sequencing work. The measured sequences were processed by BioEdit and Sequin software before they are submitted to the GenBank database.

3.7.4 Results are compared

Sequencing results were performed for the NCBI-BLAST alignment.

3.8 Culture solid

Figure 3.4 - B was selected as the purified object after the solid culture for several days

Figure 3.5 -Pcake A and end B were used for purification

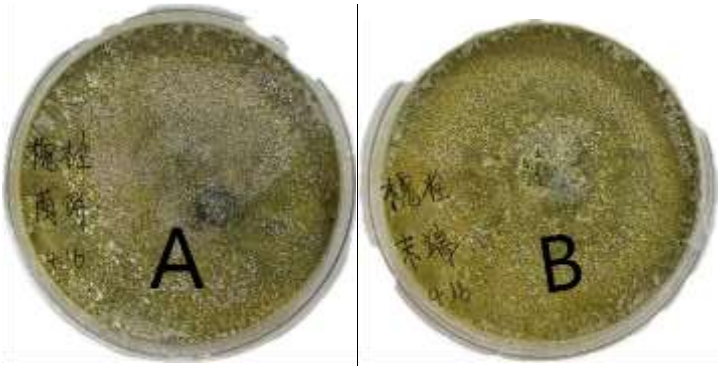

Figure 3.6 - Purification results of locust cake A and end B

3.9 liquid culture

Bottle A was placed in the incubator after four days of static culture to produce purified locust flora(Figure 3.7).

The experiment showed that the bottle B was grown for four days at 150 r/min at 30℃(Figure 3.8).

Bottle C added L tobacillus 56 in a static culture in an incubator for four days(Figure 3.9), Continued incubation produced abundant hyphae covering the surface of the medium.

Figure 3.7 - Liquid culture A / B / C

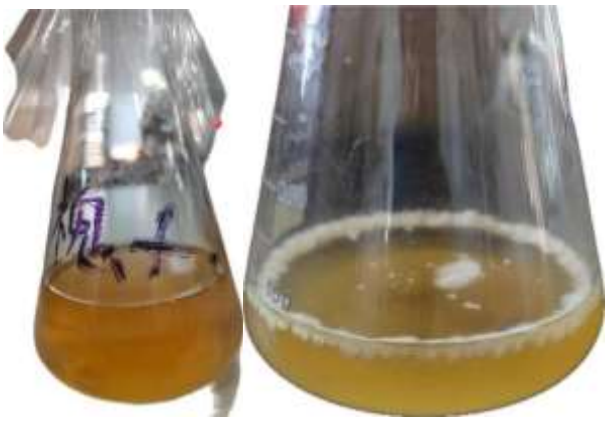

Figure 3.8 - Purified by fermentation in the static incubator of locust A

Figure 3.9 - Loclocust B shake flculture for 30℃ 150r / min

Figure 3.10 - Add a liquid static culture of C. locust C with L. reuteri 56

3.10 Data analysis

The acidity of LT C and D with L. 56 was measured by PH meter, respectively, in Table 3.5 below:

Table 3.5 - **The results of pH measurement**

Date	4.25	4.26	4.27	4.28
SJ-C PH	5.06	4.67	4.23	3.57
SJ-D PH	4.74	4.32	3.91	3.22

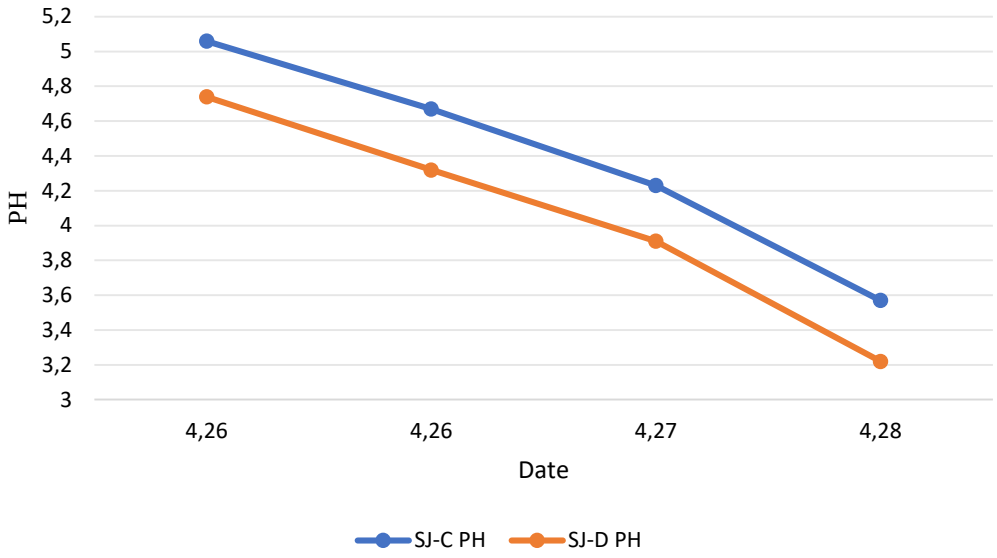

Figure 3.11 - Change of PH with fermentation time

Finally, the reduced sugar content of liquid locust C and D with L. 56 were measured by handheld refractometer, respectively, as shown in Table 3.6 below:

Table 3.6 - **Reduction sugar measurementor**

Date	4.25	4.26	4.27	4.28
SJ-C reducing sugar	3.2	3.0	3.0	2.7
SJ-D reducing sugar	3.0	3.2	3.0	3.0

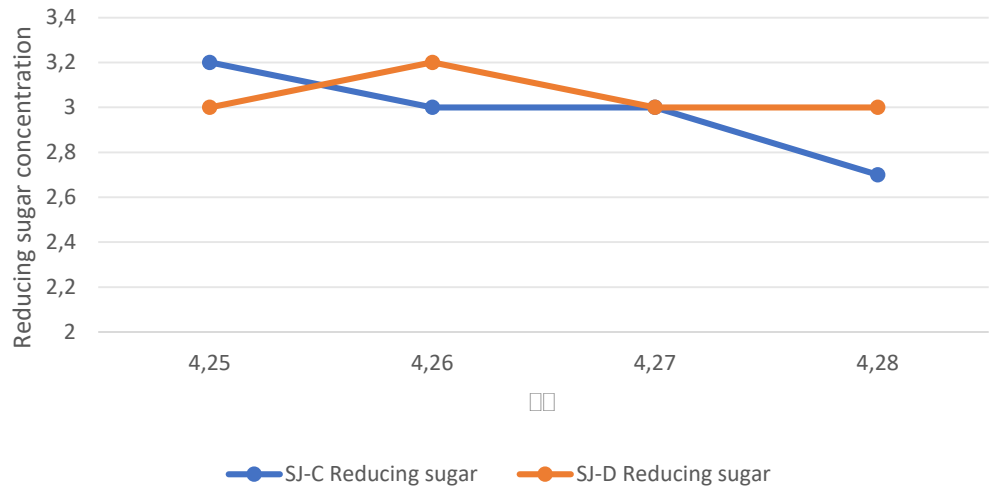

Figure 3.12 - Changes of reduced sugars with fermentation time

3.11 Alignment analysis of the sequencing sequences of the target strains

The PCR amplification products were detected by agarose gel electrophoresis and imaged under the UVP gel imaging system

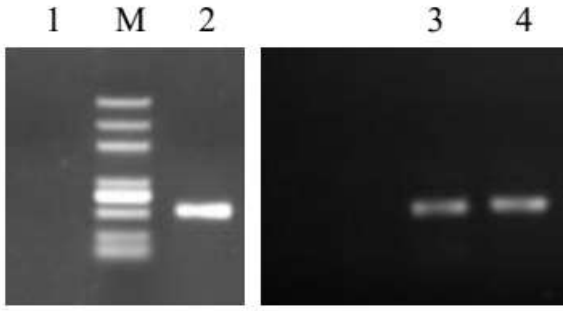

Figure 3.13 - An agarose gel electrophoresis diagram

(Note: M from top to bottom is 5000,3000,2000,1000,750,500,250,100bp.)

Table 3.7 - **The electrophoresis pore positions correspond to the samples and the primers**

Hole number	amplification primer	sequencing primer	Sample name
3	ITS1+ITS4	ITS1+ITS4	SJ-C
4	ITS1+ITS4	ITS1+ITS4	SJ-D

The nucleotide sequencing result of the ITS region of the SJ-C strain was as follows:

CCGTTCAAAACTCGATAATGATCCTTCCGTAGGTGAACCTGCGGAAGGAT

CATTATCGAGTTTTGAACGGGTTGTCTGCTGGCTCGCGAGAGCATGTGCA

CGCCTGTCTTCATCCACTCTCCACTTCTGTGCACTTTTCATAGGCCGGCT

TGTGGGTGCACGTTCGCGTGTGTCTGTAGGTCTGGTTTATGCTTTATACT

ACAAACGAATCAGTTTTAGAATGTCATACTTTGCTATAACGCAATTATAT

ACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAG

CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC

TTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGA

GTCTCATGGAATTCTCAACCTTTGACTTTGTTGTCGGAGGCTTGGACTTG

GAGGTCGTGTCGGCTCTCGTAGTCGACTCCTCTGAAATGCATTAGTGCGA

ACGTTACCAGCCGCTTCAGCGTGATAATTATCTGCGTTGCTGTGGAGGGT

ATGCTAGTGTTCGCGCTTCTAACCGTCTTCGGACAACATTTCTAAACTCT

GAGCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAAT

The sequencing peak map of the SJ-C strain is as follows:

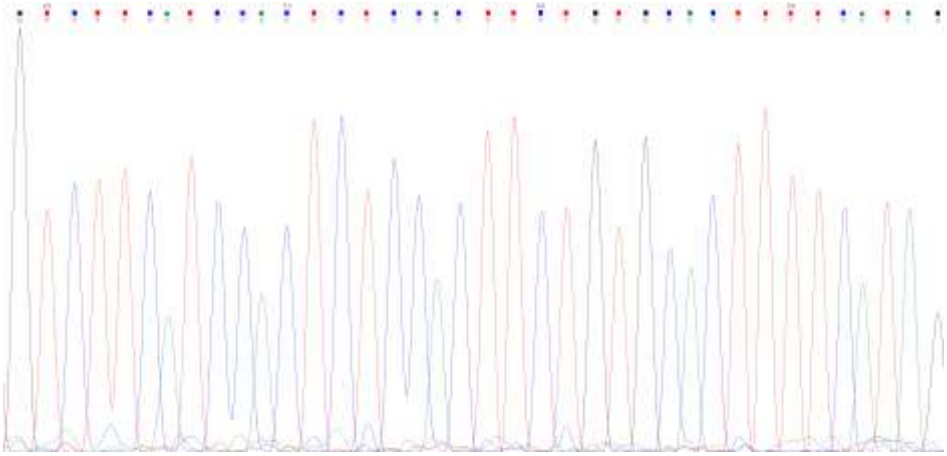

Figure 3.14 - sequencing peak map of SJ-C

The nucleotide sequencing result of the ITS region of the SJ-D strain was as follows:

ACCTGCGGAAGGATCATTATCGAGTTCTGACTGGGTTGTAGCTGGCCTTC

CGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTG

TGGGTTTACGGGTCGTGAAACGGGCTCGTTTATTCGGGCTTGTTGAGCGC

ACTTGTTTCCTGCGTTTATCACAAACTCTATAAAGTATCAGAATGTGTAT

TGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTC

TCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG

AATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATT

CCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAATCTACAAACTT

CTTATGGGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGTCCCTTTACAG

GTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTTGTC

GGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTGTTTGGGCGAGCTTC

TAACCGTCTCGTTACAGAGACAACTTTTATGACCTCTGACCTCAAATCAG

GTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGG

The sequencing peak map of the SJ-C strain is as follows:

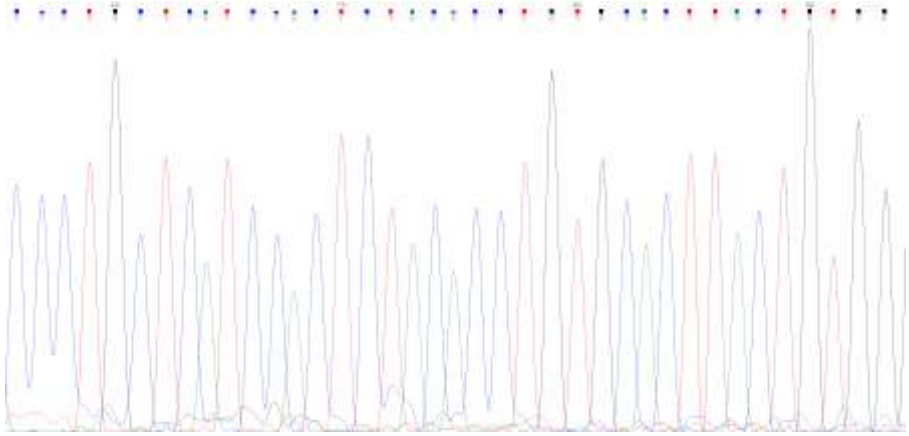

Figure 3.15 - sequencing peak map of SJ-D

The BLAST alignment was performed at the GenBank (Figure 3.16).

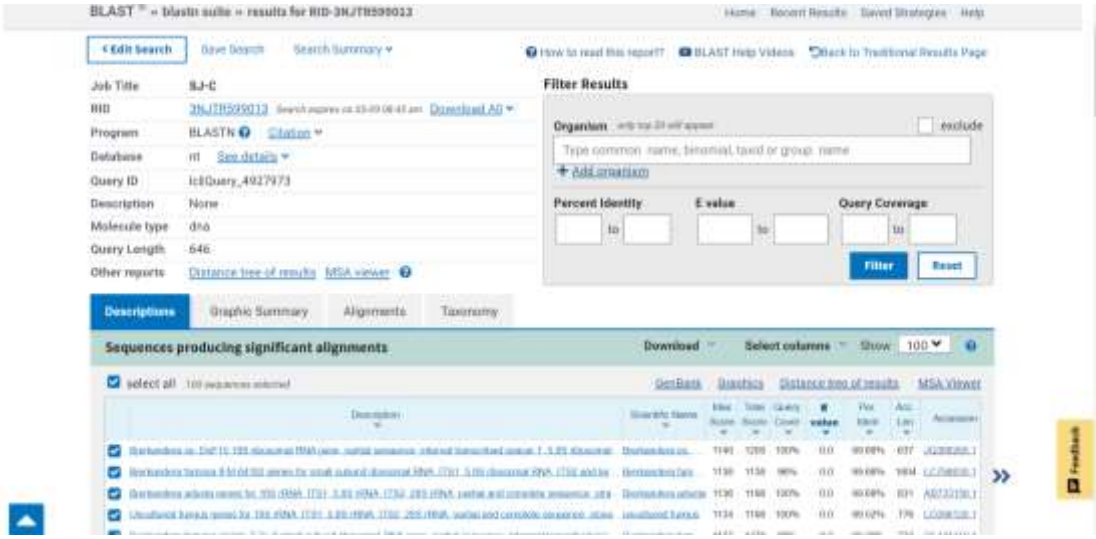

Figure 3.16 - SJ-C BLAST results of the locust ITS sequence

Figure 3.17 - SJ-C BLAST Graphic summary

It can be seen from the figure that most of the alignment sequences are the same. Red indicates that the alignment matching degree is greater than 200, while green indicates that the alignment matching degree is between 50-80.

The BLAST alignment was performed at the GenBank (Figure 3.18).

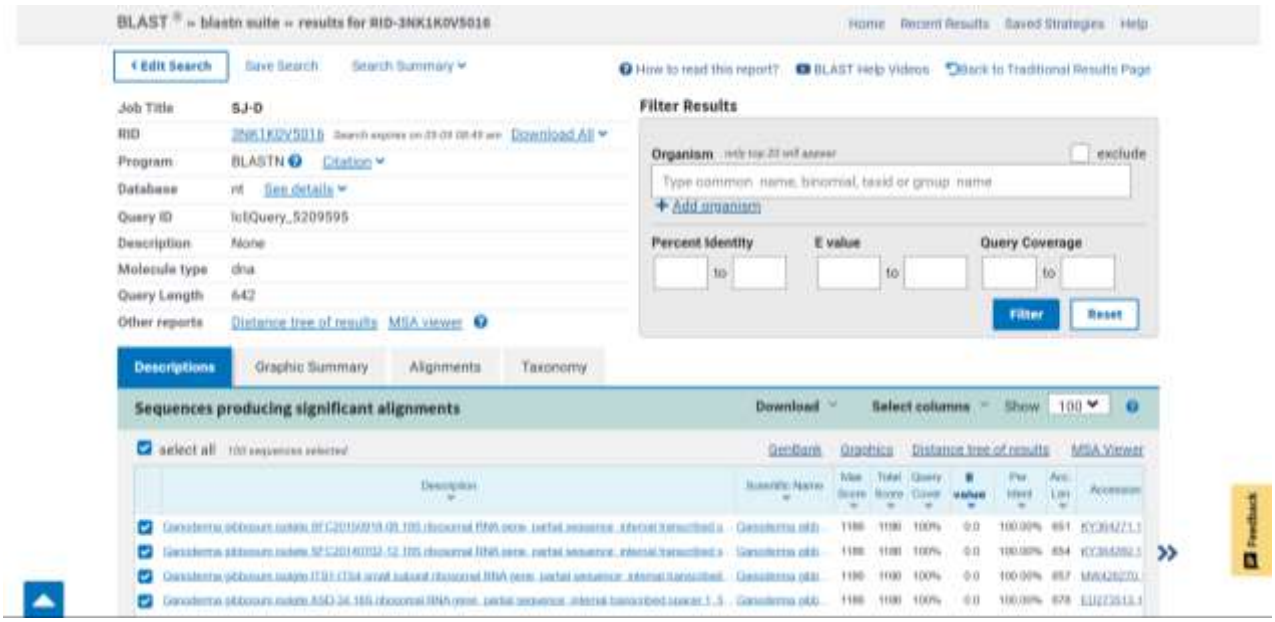

Figure 3.18 - SJ-D BLAST results of the locust ITS sequence

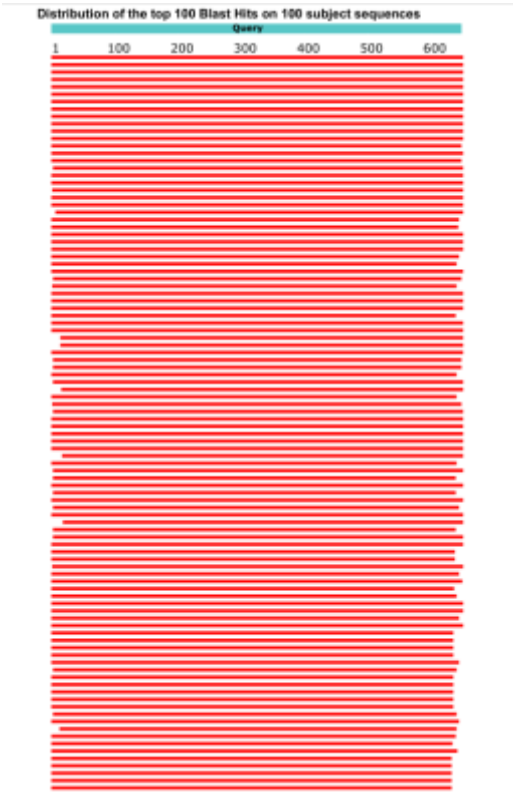

Figure 3.19 - SJ-D BLAST Graphic summary

It can be seen from the figure above that most of the comparisons are relatively similar, and red indicates that the matching degree is greater than 200,and the more red the matching degree is higher.

Different lengths of red bars indicate different lengths of base sequences.

Ten sequences with> 95% similarity to the target fragment were downloaded in GenBank, and the downloaded sequences were aligned using MEGA-X software to draw the phylogenetic tree using the adjacency method. From Figure 3.20, the closest phylogeny to SJ-C was Bjerkandera fumosa, and the SJ-C species was identified as sub-melananopella.

Figure 3.20 - Phylogenetic tree of SJ-C locust

According to Figure 3.21, the closest phylogeny to SJ-D is Ganoderma gibbosum, which identifies SJ-D species as Ganoderma lucidum.

Figure 3.21 - Phylogenetic tree of SJ-D locust

The Blast alignment result is performed as follows:

Table 3.8 **- Comparison results**

Sample name	Description	Max Score	Total Score	Query Cover	Evalue	Per.Ident	Accession
SJ-C	Bjerkandera sp. DoF15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1140 JQ38	1200	100%	0	99.68%	JQ388260.1
	Bjerkandera fumosa IFM 64182 genes for small subunit ribosomal RNA, ITS1, 5.8S ribosomal RNA, ITS2 and large subunit ribosomal RNA, partial and complete sequence	1138 LC79	1138	96%	0	99.68%	LC798830.1
	Bjerkandera adusta genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: NBRC 4983	1136	1198	100%	0	99.68%	AB733156.1
SJ-D	Ganoderma gibbosum isolate	1186	1186	100%	0	100.00%	KY364271.1

SFC20150918-08 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence						
Ganoderma gibbosum isolate SFC20140702-12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1186	1186	100%	0	100.00%	KY364260.1
Ganoderma gibbosum isolate ITS1-ITS4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1186	1186	100%	0	100.00%	MW42627.1

Conclusions to chapter 3

1.Solid-state fermentation yields Huai ear fungus, whereas liquid cultivation fosters mycelium growth. The liquid fermentation process entails the formulation of a liquid culture medium, originating from tube seed cultures. This culture is progressively upscaled, employing shake flask or fermenter apparatus to proliferate mycelial growth.

2.ITS sequence analysis serves as a pivotal molecular biology technique for fungal characterization. ITS, denoting Internal Transcribed Spacer, encompasses ITS1 and ITS2 regions. Characterized by moderate length, these regions facilitate comparative analysis. Notably, ITS sequences demonstrate intraspecific uniformity yet interspecific variability, rendering them instrumental in fungal classification and strain delineation.

3.Agarose gel electrophoresis stands as a proficient technique for the separation and extraction of high-molecular-weight DNA strands. Within agarose gel matrices, DNA molecules undergo differential migration rates contingent on their respective molecular masses, a principlefundamental to their utility in PCR product detection.

CONCLUSIONS

1. In recent years, with the development of biotechnology and the progress of science and technology, people have never stopped the development and utilization of locust fungus and its products, and the heat is high, people have been looking for a more productive fermentation technology.The experiments in this paper aimed to sequence and compare the two locust strains, optimize the fermentation temperature and analyze their products. In addition, some difficulties and problems encountered in the experiment need to be explored and solved. For example, for example, the liquid locust was not incubated in the static culture and at 30℃ 150 r/min.

From the perspective of the ph measurement results, the results more stable gradually decline, present weak acid state, the experiment only determined the acidity of the fermentation broth, did not further explore the influence and the cause of the PH, for example, in the fermentation fermentation by the environment, temperature and fermentation time changes, the regularity of acidity decline research has not been further discussed and research, so understand the influence of acidity on fermentation need further experiments.

According to the detection results of the reduced sugar, the reduced sugar decreased relatively steadily with the increase of the number of fermentation days, which could not fully reflect the performance in the fermentation process. The result was not discussed and analyzed, and the conclusion is not perfect. There is the situation of reducing sugar rising, which may be caused by the formation of miscellaneous bacteria contamination in the fermentation process.

From the results of comparison analysis, the phylogenetic tree of SJ-C species drawn by MEGA shows the relationship between SJ-C and Bjerkandera fumosa, and is identified as Bjerkandera fumosa; while the phylogenetic tree of SJ-D shows its close relationship to Ganoderma gibbosum, which is identified as Ganoderma.

2. The efficacy of locust fungus is numerous and the side effects are small, and the application of locust fungus is also constantly explored and launched. It can be found in health care products, medicines and even cosmetics, which marks the

indispensable position of locust fungus in our production and life. Due to the high nutritional value of locust fruiting body, people have been looking for ways to improve the shape of the fruiting real economy and the quality of locust fungus, hoping to make full use of locust and its products. The identification of locust bacteria is the first step to make full use of locust bacteria, where modern molecular biology plays an important role in combining with traditional identification methods. Targeted cultivation of fruiting bodies through different media formulations and cultivation methods of locust bacteria can improve the yield and quality of locust bacteria and play the maximum effect of locust bacteria. This is also the most critical work to enable the comprehensive use of a large area. Therefore, the study of the fermentation process is important for the subsequent development of locust fungus and its products.

3. From this point of view, the development and utilization of locust fungus has great potential. In the process of continuously improving the quality and economic value of its products, it will gradually form a scale and industrialization, promote social and economic development, and benefit the well-being of human beings.

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