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 **Research on the extraction of active ingredients from *Scutellaria baicalensis* by probiotics fermentation**

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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APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor,
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Olena MOKROUSOVA
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«___»________________2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Qin Yuhang

1. Thesis topic **Research on the extraction of active ingredients from *Scutellaria baicalensis* by probiotics fermentation**

Scientific supervisor Olga Andreyeva, Dr. Sc., 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	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 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 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

Student ____________ Qin YUHANG

Scientific supervisor ____________Olga ANDREYEVA

SUMMARY

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As the unique medicines of Chinese medicine, Chinese herbs are widely used in the fields of medicine, biological research and development, and food, due to their natural origin, rich variety, low toxicity and side effects, and mild and long-lasting therapeutic effects. With the development of modern biotechnology, modern herbal fermentation technology has been formed and continuously improved. Studies have shown that through modern herbal fermentation technology, the content of active ingredients in herbal medicines can be effectively increased and new beneficial ingredients can be produced, which provides a new way for the further utilization of herbal medicine resources. Probiotics are a class of microorganisms that are beneficial to the host, and herbal medicines fermented by probiotics have the advantages of increasing the active ingredients of medicines and reducing the toxic side effects. *Scutellaria baicalensis*, a commonly used Chinese herb in China, has pharmacological effects such as antioxidant, antibacterial and anti-inflammatory. In this experiment, three strains of lactic acid bacteria were used to ferment *Scutellaria baicalensis* powder and tested for antioxidant as well as bacteriostatic indexes. The results indicated that *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus delbrueckii* reached their maximum antioxidant and bacteriostatic indexes measured at 72 h of fermentation, which were DPPH scavenging: 99.02%, 95.4%, and 94.5%, hydroxyl radical scavenging: 77.5%, 77.5%, and 84.9%, and the diameter of the circle of inhibition:14.5 mm, 13.75 mm, and 14.1 mm, respectively.

Keywords: modern herbal fermentation technology; Lactic acid bacteria; Scutellaria baicalensis; antioxidant; bacteriostatic

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INTRODUCTION

Chinese herbal medicine is widely used in medicine, biological research and development, food and other fields because of its natural source, rich variety, small toxic and side effects, mild and lasting therapeutic effect. With the development of modern biotechnology and the extensive research and utilization of microorganisms, modern Chinese herbal fermentation technology has been formed and improved. Modern Chinese herbal fermentation technology provides a new way for the further innovation and utilization of Chinese herbal medicine and its related products. As a kind of widely used microorganism, probiotics are closely related to human life. The probiotic fermentation of Chinese herbal medicine has the advantages of improving the effective ingredients of medicine and reducing the toxic side effects. As a commonly used Chinese herbal medicine, Scutellaria baicalensis has pharmacological effects such as anti-oxidation, antibacterial and anti-inflammatory. In this experiment, three strains of lactic acid bacteria were used to ferment *Scutellaria baicalensis* powder, and its antioxidant and antibacterial indexes were tested.

The relevance of the topic is Probiotic Fermented *Scutellaria baicalensis*.

The purpose of the study is the Genome sequencing of existing lactic acid bacteria strains in the laboratory and selection of three strains for *Scutellaria baicalensis* fermentation, and to test the antioxidant and bacteriostatic indices of the fermentation broths by relevant methods.

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The object of the study is Probiotic fermentation of *Scutellaria baicalensis* and fermentation broth related indicators

The subject of the study is Fermentation of *Scutellaria baicalensis* by probiotics and testing of antioxidant and bacteriostatic indexes of Scutellaria baicalensis fermentation broth by relevant methods

Research methods:

I：Genome extraction and testing of existing strains in the laboratory to confirm the three strains used for fermentation.

II：Grinding of herbal raw materials → autoclaving and drying → probiotic strains → activation and transfer → inoculation of powder → shaking and homogenization → fermentation.

The scientific novelty: Probiotic fermentation may improve the extraction efficiency, bioavailability and safety of the active ingredients of Chinese herbal medicine, and may also produce some new active metabolites, which provides a new idea and method for the application and development of *Scutellaria baicalensis*.

The practical significance of the results obtained is Probiotic fermentation may improve the extraction efficiency, bioavailability and safety of the active ingredients of Chinese herbal medicine, and may also produce some new active metabolites, providing new ideas and methods for the further application and development of Chinese herbal medicine.

The structure and scope of the dissertation. A Qualification thesis consists of an introduction, three chapters, a conclusion, and a list of references (56 titles).

CHAPTER 1
LITERATURE REVIEW

1.1 Introduction to herbal medicine

Herbal medicines are parts of plants with medicinal value, such as roots, stems, leaves, flowers, fruits, etc. They are unique medicines used in Chinese medicine for the prevention and treatment of diseases, which marks a significant difference between Chinese medicine and other fields of medicine. These medicines have a variety of active ingredients, such as flavonoids, polysaccharides and alkaloids. Also, herbs contain a variety of pharmacological effects, such as antioxidant, immune system modulation [1-3], antibacterial [4-5], and anti-inflammatory.

Due to their natural origin, wide variety, low toxicity and side effects, mild and long-lasting therapeutic effects, and the ability to fundamentally regulate and improve the physical condition of patients, Chinese herbal medicines have been more and more widely used in human medicine, biological research and development, as well as in the field of food and other areas. In order to improve the preventive and therapeutic effects of Chinese herbal medicine, it is usually necessary to carry out simple processing and extraction of Chinese herbal medicine. Traditional herbal extraction processes mainly include decoction, cold maceration and alcohol precipitation. However, these extraction methods often require a long extraction time, relatively low extraction efficiency, complex operation procedures and large impurity content [6], making the active ingredients in Chinese herbal medicines cannot be fully utilized.

1.2 Herbal Fermentation Technology

Ancient working people in China used to use fermentation technology for brewing wine for more than 4,000 years, and on the basis of this technology fermented vinegar, sauces, and foods such as tempeh. Around the Han Dynasty, more than 2,000 years ago, the method of using fermentation technology for herbal medicine concoctions appeared, resulting in the earliest fermented traditional Chinese medicines, i.e., fermentation quats, including red quat, half-summer quat, and six-spirit quat, etc. [7-8]. Traditional Chinese herbal medicine fermentation technology is a method of mixing Chinese herbs with auxiliary materials, placing them at a certain temperature and humidity, and using molds to make them foaming and moldy, as well as changing the medicinal properties of the original medicine to produce a new medicine. As the strains used in traditional fermentation come from the air or rely on other ways to inoculate the strains, the types and numbers of strains will be affected by the environment, such as soil, temperature, humidity, oxygen concentration, and weather changes, which will in turn affect the results of fermentation. In addition, the traditional fermentation process mainly relies on the operator's personal experience to judge and control, the lack of single-variable regulation and related indicators of the test, with a greater subjectivity, there are large errors, so the quality of the fermentation product fluctuations in the quality of the product, repeatability is poor [9], the active ingredients cannot be effectively utilized.

In the continuous development of modern biotechnology and the continuous updating and iteration of bioengineering equipment, the modern herbal fermentation technology combined with modern bioengineering and technology is formed and developed. And microorganisms also show more and more important role. Microorganisms are tiny but closely related to human beings, mainly involved in the fields of food, medicine, environmental hygiene and cosmetics. The strains required for modern herbal fermentation are engineered bacteria based on purification of existing fermentation strains and improved by genetic means, cell engineering, etc. [10]. The key step in the process of herbal fermentation is the selection and breeding of high-quality strains. Modern herbal fermentation has developed single strain purification fermentation and composite strain purification fermentation. In addition, compared with the traditional herbal fermentation, the improvement of technology and the advanced instrumentation make the modern fermentation process reduce the influence of human subjective factors, the efficiency of strain transformation becomes more efficient and controllable, and the quality of fermentation products is more stable and specialized.

1.2.1 History and Current Status of Herbal Fermentation Technology

Mono-strain fermentation is a fermentation process that utilizes only one specific strain of microorganisms to obtain a specific product or compound. The strains commonly used in modern herbal fermentation processes are bacteria and fungi. Bacteria are used in the fermentation process due to advantages such as the ability to produce various types of enzymes, usually have a faster growth rate which increases productivity, the ability to utilize different organic substances as substrates for fermentation, and the ability to produce many different types of products. Bacteria commonly used in fermentation include *Escherichia coli*, one of the most commonly used bacteria, commonly used in biotechnology and industry, such as recombinant bioproteins and biodiesel; *Lactobacillus*, a group of Gram-positive bacteria mainly used in lactic acid fermentation to produce lactic acid and other *Lactobacillus* -fermented products such as yoghurt; and *Bacillus cereus*, has the ability to efficiently stop the growth of pathogenic microorganisms, to achieve the effect of bacteriostasis, production of antibiotics, etc. [11]. Keilin Yang [12] showed through his research that *Lactobacillus plantarum* fermentation can maximize both ginkgolide B and total phenol content in ginkgo juice, while ginkgo fermentation samples play an important protective function for vascular endothelial cells. This is of great significance for the further utilization of the resources of ginkgo. The study of Qu Qingsong et al. [13] showed that after fermentation of ginseng by lactobacilli, the content of each ginsenoside in the fermentation system increased to some extent, among which ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, ginsenoside Rc, ginsenoside Rd increased by 69%, 62%, 73%, 34%, 64%, respectively, and a new ingredient was produced by the fermentation. The study of Wen Li et al. [14] showed that the protease hydrolysis and antioxidant capacity of chickpea fermented by *Bacillus subtilis* was significantly improved, which makes fermented chickpea available for novel food development.

Fungi have the advantages of strong enzyme production, effective and thorough decomposition of substrates, relatively relaxed culture conditions, and the production of many different types of substrates through the selection of different strains of fungi and the adjustment of fermentation conditions, which also often make them used as engineering bacteria in the fermentation process. Examples include *Saccharomyces cerevisiae*, *Aspergillus*, etc. Wang Yanping et al. [15] conducted a study on solid-state fermentation of fresh *Codonopsis pilosula* by yeast and found that after fresh *Codonopsis pilosula* was fermented by yeast, the content of polysaccharides was increased and the content of total sugars and oligosaccharides was decreased. At the same time, the fermentation product, 95% ethanol extract, showed high activity in scavenging DPPH, superoxide anion, and hydroxyl radicals. Liu Youzhi et al.[16]carried out liquid fermentation of buckwheat by *Aspergillus niger*, the study showed that the fermented buckwheat soluble dietary fiber increased, dietary fiber water-holding capacity, oil-holding capacity and swelling capacity have increased, regulating the microbial capacity in the intestines of mice on a high-fat diet has been enhanced, and the bioactive components have also increased.

In addition to the commonly used bacteria and fungi, there is also a type of medicinal fungus. Dual-use fungi refer to a group of fungi that have both medicinal and food values. These fungi are usually rich in biologically active substances, have certain medicinal value, calm medicinal power, less adverse reactions, and also have certain nutritional and health functions in the field of food. Both cater to the current social demand for medicinal diets, food therapy and other aspects, but also fit the future direction of the development of traditional Chinese medicine [17]. At present, common medicinal and food mushrooms mainly include Ganoderma lucidum, tea tree mushroom, matsutake mushroom, monkey head mushroom and so on. Li Fang et al. [18] showed through their research that Ganoderma lucidum polysaccharide (GLP) has the effect of inhibiting the proliferation of cancer-associated fibroblasts (CAFs), inducing apoptosis of CAFs, inhibiting the activity of CAFs, and possibly inhibiting the metastasis of intestinal cancer, and speculated that its mechanism of action may be related to the TGFβ1/Smad2 signaling pathway. Dual-use bacteria in modern society is in a booming stage, its application market is very broad, the medicinal value is also to be developed, of course, its technological innovation, product quality and safety issues also need to be improved and developed.

1.2.2 Application of Herbal Fermentation Technology

1.2.2.1 Composite strain fermentation

Composite strain fermentation, as the name suggests, is the process of fermenting herbal raw materials using a variety of different microbial strains either simultaneously or sequentially. Compared with single-strain fermentation, composite strain fermentation has a wider combination of strains, which can utilize the synergistic effect between multiple strains and improve the efficiency of the fermentation process and the quality of the products. Yue Yangyang [19] by studying the effect of co-fermentation of Saccharomyces cerevisiae and Lactobacillus plantarum on wolfberry wine, showed that the co-fermentation increased volatile substances and metabolites of lactic acid bacteria, and at the same time produced a variety of esters and alcohols, which made the aroma of wolfberry wine more complex and mellow, and the overall is obviously superior to the single-bacteria fermentation.

Dong Yu et al. [20] showed that on the 45th day of fermentation, the combination of *Bacillus subtilis* and *Aspergillus niger* addition fermentation of sweet sorghum straw in fermentation quality and prolonged aerobic stability, etc. due to the monoculture fermentation group. De Vuyst L. et al. [21] in the fermentation of cocoa showed that yeast can ferment glucose from the cocoa pulp into ethanol, carry out the decomposition of pectin and produce flavor compounds; *Lactobacillus* can provide microorganisms with a stable fermentation environment, provide lactic acid as an indispensable source of carbon in the acetic acid bacteria, and contribute to the flavor of cocoa and chocolate by producing sugar alcohols, organic acids, etc., while acetic acid bacteria can oxidize ethanol to acetic acid, which penetrates bean leaves and prevents seed germination.

1.3 Characteristics of modern fermentation technology

1.3.1 Effects on the pharmacological effects of herbal medicines

(1) Antimicrobial Guo Lijun [22] selected *Lactobacillus rhamnosus*, *Lactobacillus plantarum, Bacillus coagulans* and *Bacillus subtilis* were selected to ferment three groups of traditional Chinese medicine compound. The results of the study showed that one group of Chinese herbal compounds consisting of umeboshi, erythrose, licorice, schizandra and dihu were effective in the treatment of Escherichia coli infection in mice. In addition, the fermentation broth of this group of herbal compounds fermented by *Lactobacillus rhamnosus* showed the best therapeutic effect against E. coli in mice. Moon K. et al. [23] fermented *Salvia divinorum* root with *Aspergillus miltiorrhiza* at 25 °C for three weeks, extracted the non-fermented (SME) and fermented (SMBE) roots with 70% ethanol then fractionated them with organic solvents respectively, and the results showed that the antimicrobial activity of SMBE was twice as much as that of SME.

(2) Lowering blood lipids Qin Ling Caiet al. [24] used *Bacillus subtilis* and *Lactobacillus plantarum* to ferment natto yogurt and act in a mouse model of hyperlipidemia. The results showed that after consuming natto yogurt for five weeks, mice showed significant improvement in body weight, fat, liver weight, and decreased serum TG, TL, and LDL levels. Natto yogurt significantly reduced the area of hepatic fat infiltration and the number of lipid droplets. Natto yogurt may inhibit fatty acid synthesis and enhance the inhibition of fatty acid anabolic metabolism by regulating the expression of PPARα, PPARγ, CD36, and FAS in the liver, thus playing a role in lowering blood lipids. Huijie et al. [25] used *Lactobacillus, Saccharomyces cerevisiae* and *Acetobacter* as fermentation strains, and *Lotus* leaf, Chenpi and Hawthorn as substrates for the preparation of *Lotus* leaf formula herbal enzyme. The results showed that the binding rate of *Lotus* leaf formula with sodium glycochenolate after

fermentation was 63.53%, and with sodium taurocholate was 63.51%, which were significantly higher than the binding rate before fermentation. This also indicates that the Lotus leaf formula herbal enzyme has better in vitro hypolipidemic efficacy. Shouquan Ku [26] uses *Ginseng* as the medicinal fermentation substrate and the medicinal fungus *Aspergillus erythrorhizus* was used for two-way solid fermentation, and then an experimental hyperlipidemia mouse model was established. The results showed that Monacolin K, an active ingredient with hypolipidemic efficacy, was detected in the fermentation products, while the positive control group, the high-dose administration group and the medium-dose administration group all significantly or highly significantly reduced the contents of TG, TC and LDL-C in hyperlipidemic mice, and the medium- and high-dose groups showed a better tendency to lower the contents of TG, TC and LDL-C in the positive control group However, there was no significant difference. This indicated that the fermentation product of Aspergillus rubra-ginseng had good hypolipidemic efficacy. Yang Jingyun et al. [27] used hawthorn, zedoary and cassia seeds as fermentation substrates and laboratory-screened lovastatin-producing *Aspergillus erythropolis* as strains for solid-state fermentation, and the results showed that compared with rice erythropolis, the traditional Chinese medicine-erythropolis fermentation product contained more active ingredients and the yield of lovastatin was also increased by 42.27%. The study showed that hawthorn, zedoary and cassia seeds could not only significantly increase the amount of lipid-regulating active ingredients in addition to the amount of active ingredients in traditional Chinese medicine after solid-state fermentation by *Aspergillus oryzae*, which is of great significance as a guide for the future research and development of lipid-lowering drugs.

(3) Regulation of intestinal flora Chang Aixin et al. [28] showed that the extract of yellow essence increased the abundance of thick-walled *Bacillus phylum,* decreased the abundance of deformed bacillus phylum, increased the relative abundance of bifidobacterium genus, and significantly decreased the relative abundance of fusiform streptococcus bacteria after simulation of in vitro digestion and it had a certain

modulating effect on the intestinal flora. Li Rong et al.[29] [29] investigated the effects of fermented polysaccharides on the changes of intestinal flora and short-chain fatty acids in mice and their relationship with the level of intestinal inflammation and the expression of barrier proteins, it was shown that fermented LBP was able to enrich the intestinal *Bacillus dubliniensis* and *Bacteroides graminicola* spp. in mice, decrease the abundance of *Enterobacter* spp. and *Escherichiaceae-Shigella* spp., and was also able to significantly reduce the level of intestinal inflammation in mice, and improve the structure of the colonic tissues. Liu Gongxiao et al. [30] Fermentation inoculation of *Strychnos cinerea* with *Saccharomyces cerevisiae* and establishment of a rat model of T2DM combined with hepatic injury by continuous feeding of high-fat and high-sugar feed plus streptozotocin injection. The results showed that *Strychnos cinerea* fermentation significantly increased the diversity of the intestinal flora of the rats and up-regulated the ratio of *Bacillus cerevisiae*/*Bacillus pseudomallei* to maintain a stable structure of intestinal flora; it also increased the abundance of probiotic bacteria such as *Lactobacillus* spp. and reduced *Escherichia-Shiegleria* spp. in the intestine. The results of this study showed that the abundance of probiotics such as *Lactobacillus* and *Escherichia-Shigella* in the intestines increased and the abundance of inflammatory microorganisms such as *Escherichia-Shigella* decreased, which strengthened the intestinal defense function.

Liu He et al. [31] conducted experiments on mice with sodium ceftriaxone-induced intestinal mucosal barrier damage model using fermented *Ganoderma lucidum* substrate fermentation broth from *Bacillus subtilis*. The results showed that the mice recovered their body weight after the fermentation solution treatment, and the swelling of the colon was improved; the histopathological damage of the colon was reduced, and the infiltration of inflammatory cells was significantly reduced; serum IL-10 increased significantly and LPS, TNF-α, and IL-6 decreased significantly compared with the model group; in addition, the excessive up-regulation of the T-cell ratio and the intestinal flora dysbiosis caused by ceftriaxone were also ameliorated. It suggests that *Bacillus subtilis-Linoderma lucidum* substrate fermentation solution can effectively improve the intestinal flora dysbiosis and regulate the intestinal mucosal barrier function in mice.

(4) Anticancer Wei Han et al. [32] extracted polysaccharides (GSRBPs) from *Ganoderma lucidum* - full fat rice bran (GS-FRB) and *Ganoderma lucidum* - defatted rice bran (GS-DRB) fermentation products. The structural information of GSRBPs was investigated by HPLC analysis. The in vitro antitumor activity of H1299 NSCLC of GSRBPs was investigated by MTT method, which showed that all polysaccharides contained two fractions, GSFPS-1 and GSFPS-2, and the IC50 maxima and minima of the RBS and GSRBPs in the in vitro study were found to be GS-DRB-13 (60.63 μg/mL), GS-DRB-11 (40.62 μg/mL), and in the in vivo study, the maximum and minimum H1299 NSCLC inhibition rate (InRa) of RBS and GSRBPs were GS-DRB-11 (86.81%), GS-FRB-7 (27.87%), respectively. It indicated that GSFPS-2 area percentage was negatively correlated with IC50 and positively correlated with InRa. This implies that GSFPS-2 has much higher anti-tumor activity than GSFPS-1. This is instructive for the future development of potential new drugs for non-small cell lung cancer. Hyun-Dong Cho et al. [33] investigated the anticancer effects of unfermented (SEE) and fermented silkworm larvae ethanolic extract (FSEE) on HepG2 human hepatocellular carcinoma cells. The study showed that FSEE was able to lead to apoptosis of HepG2 cells as compared to SEE, which was characterized by G0/G1 phase cell cycle arrest, DNA fragmentation and apoptotic vesicle formation. In addition, FSEE significantly up-regulated pro-apoptotic proteins and down-regulated anti-apoptotic proteins in HepG2 cells. The results suggest that solid-state fermentation of silkworm larvae by *Aspergillus glabrata* strongly enhanced cysteine asparaginase-dependent and non-dependent apoptotic pathways in human hepatocellular carcinoma cells by modulating secondary metabolites.

Fan Haineng et al. [34] investigated the effects of noni fruit fermentation broth on proliferation and apoptosis of hepatocellular carcinoma cells *in vitro*. The results showed that the proliferation inhibition rate and apoptosis induction rate of the fermentation solution on hepatocellular carcinoma cells Bel7402 varied at different concentrations and increased with the increase of concentration, and protein blotting experiments showed that the expression of Survivin gene in the treated hepatocelluLar carcinoma cells Bel7402 was significantly lower than that of the control group, which indicated that the noni fruit fermentation solution might induce hepatocelluLar carcinoma cells in vitro by inhibiting Survivin gene expression to induce apoptosis in hepatocelluLar carcinoma cells in vitro. Yang Yingge et al. [35] optimized the solid bidirectional fermentation process of *Ganoderma lucidum-Astragalus membranaceus* dregs and determined the in vitro antitumor activity of bacterial plasmic polysaccharides on human intestinal cancer HCT116 cells by MTT method. The results showed that the in vitro inhibition rate of mucopolysaccharides (mannose, rhamnose, glucose, arabinose and galactose) exceeded 85% when the concentration of mucopolysaccharides (mannose, rhamnose, glucose, arabinose and galactose) exceeded 40 mg/mL, and the mucopolysaccharides had a good inhibitory effect on human intestinal cancer HCT116 cells transplanted into nude mice when they were continuously orally gavages for 18 d twice a day (equivalent to the dose for human use). Inhibitory effect.

1.3.2 Effects on active ingredients of traditional Chinese medicine

(1) Enhancement of the content of active ingredients Yang Jilin [12] added Lactobacillus plantarum NJBC17, which has the function of biotransforming ginkgolide B, to ginkgo biloba juice for fermentation, the active ingredients in ginkgo biloba juice were promoted to biotransform into ginkgolide B, which further increased the ginkgolide B yield. The results showed that the contents of ginkgolide B and total phenols in fermented ginkgo juice were 1.30 times and 1.07 times higher than those in unfermented and sterilized ginkgo juice, respectively, and the contents of ginkgolide B and total phenols in fermented ginkgo juice were maximized by the synergistic induction of Lactobacillus plantarum NJBC17 with the use of 0.02 mg/m L of $MgCl_2$, 1 mmol/L of salicylic acid, and 0.75% of pyruvate, and the contents of ginkgolide B and total phenols were maximized. The contents of ginkgolide B and total phenols were

1.55 and 1.36 times higher than those of uninduced fermented ginkgo juice, and 2.02 and 1.45 times higher than those of unfermented ginkgo juice, respectively. It was analyzed that both *Lactobacillus plantarum* NJBC17 fermentation and synergistic induced fermentation regulated terpene biosynthesis and lipid metabolism pathway in ginkgo juice. Yan Bin [35] used *Lactobacillus plantarum* to ferment the extract of *Astragalus mongolite* and analyzed the metabolites before and after fermentation showed that the contents of more than 20 metabolites, including dicotyloside Ic, bitter amygdalin, catalpol, and isozygous prenylactone, were significantly increased after fermentation, and that the fermentation products reduced the accumulation of lipid droplets in Hep G2 cells; the inhibitory ability of α-glucosidase activity and the ability to inhibit DPPH radical, hydroxyl radical, and ABTS radical scavenging capacity oxidative capacity were also significantly enhanced.

Yin Jiaquan et al. [37] conducted solid-state fermentation of Luo Han Guo pomace using *Saccharomyces cerevisiae* and used relevant methods to detect functional components, and the results showed that functional components such as saponins, polyphenols, total flavonoids, and crude polysaccharides all showed a tendency to increase and then decrease during the fermentation process, and the contents reached the maximum of 1.42, 2.37, 2.53, and 1.90 times that of the non-fermented group, respectively. Moreover, the fermentation process obviously improved the antioxidant activity of the functional components, which is of great significance for the further recycling of Luo Han Guo pomace.

(2) Reducing the toxic side effects of drugs Duan Qixuan et al. [38] established a model of SD rat specific hepatotoxicity by two-way solid fermentation of *Ganoderma lucidum bacteria* with He Shouwu, and explored the effect of bioconversion of *Ganoderma lucidum* bacteria on the specific hepatotoxicity of He Shouwu by using HPLC and other methods. The results showed that compared with the control group, the model group had no obvious pathological changes and no liver injury was observed. And with the decrease of the content of stilbene glycoside, the PMEE *Ganoderma lucidum* transformed group showed a tendency to reduce the degree of liver injury, and

basically, no hepatocelluLar injury was observed in the group with 100% stilbene glycoside transformation rate. It indicated that *Ganoderma lucidum* was able to reduce the specific hepatotoxicity of He Shou Wu through biotransformation, and it was hypothesized that the toxicity-reducing effect was related to the reduction of the content of stilbene glycosides in He Shou Wu. The herb, also known as *Iron Flower*, *Epiphyllum*, and *Black shunpian*, has the ability to restore yang and rescue the rebelliousness, and to improve the health of the body. It has the functions of returning yang to save the reverse, tonifying fire to help yang, dispersing cold and relieving pain, etc. However, it has a greater toxicity. However, *Epiphyllum* has a high toxicity, and excessive or improper consumption may cause poisoning or even threaten life.

Zhang Yuqing [39] used *Schizophyllobacterium* to conduct solid and liquid fermentation biological transformation of raw aconite through screening and determined the optimal fermentation conditions. The alkaloid content of the biotransformed epiphyllum was detected by HPLC method after recovery. The results showed that the content of the toxic component of the biotransformed epiphyllum in the solid-state fermentation was much lower than the standard 0.0019%, and the content of the active component of the monoester alkaloids was higher than the standard 0.0352%, and the toxicity experiments of animals showed that there was no relevant poisoning symptom of the animals in the biotransformed epiphyllum group, which indicated that the toxicity of the biotransformed epiphyllum group was significantly reduced. Through liquid fermentation, the content of bi-ester alkaloids in the bioconverted epiphyllum group was lower than the lowest detection index, and the content of mono-ester alkaloids was slightly higher than the standard of 0.013%, which also reduced the toxicity significantly.

As a traditional Chinese medicine, the dose at which the therapeutic action of Lei Gong Teng takes effect is almost equal to its toxic dose. Ho Luan Cherry et al. [40] utilized *Ganoderma lucidum* for bidirectional solid-state fermentation of *Rehmannia glutinosa* and investigated its toxicity-reducing effect through the related detection of *Lingreiomycetes* produced during the fermentation process. The results showed that only *Lingreiomycetes* plasmid (N2-G30) at a mass concentration of 3.75 μg/mL inhibited the release of TNF-α and IL-6 pro-inflammatory factors from lipopolysaccharide-induced mouse RAW264.7 cells. In addition, it inhibited the proliferation of human normal hepatocytes L02.LC-MS analysis showed that the content of active ingredients such as tretinoin and tretinoin erythropoietin increased and the content of tretinoin-like ester A decreased after fermentation, which may be related to the attenuating and holding effect of the Lingreiomycetes plasmodiales.

(3) Generation of new active ingredients Xueyue Tai et al. [41] utilized *Lactobacillus bulgaricus* to ferment ginseng to increase the content of ginsenosides and to study the type and content of fermentation-converted ginsenosides. The results showed that seven saponins, Re, Rg1, Rb1, Rb1, Rc, Rb2, Rd, and Rh1, were detected in ginseng before fermentation, but nine saponins, Re, Rg1, Rb1, Rc, Rb2, Rh1, Rd, R-rg3, and CK, were detected after fermentation, as determined by high performance liquid chromatography.

Among them, the contents of saponins Re, Rg1, Rb1, Rc, Rb2 and Rd decreased significantly, and the contents of saponins Rh1, R-rg3 and CK increased significantly. It indicates that some ginsenoside types were transformed from common saponins to rare saponins during the fermentation process. It was hypothesized that the transformation pathway might be three pathways: ginsenoside Rb1/Rb2 was first transformed into Rd and then into R-rg3; Rb1/Re/Rg1 was transformed into Rh1, and Rb1/Re was transformed into CK.

1.4 Overview of probiotics

Probiotics are a group of microorganisms that are beneficial to the health of the intestinal tract, often referred to as "friendly bacteria". They are found mainly in the human digestive tract, especially in the colon and small intestine, and not only can they help maintain the balance of beneficial and harmful bacteria in the intestinal tract, promote the normal function of the digestive system, strengthen the immune system, but may even have a positive impact on mental health. Common probiotics mainly include *Lactobacillus*, *Bacillus*, B*ifidobacterium*, etc. Probiotics can be consumed through dietary intake, such as fermented dairy products (e.g. yogurt, yogurt drinks, fermented cheeses, etc.), sauerkraut, pickles, etc., or through probiotic supplements.

1.4.1 Main functions of probiotics

(1) Promote the digestion and absorption of nutrients Probiotics can participate in the digestion of nutrients in the intestinal tract by synthesizing digestive enzymes, together with the digestive enzymes that the animal body itself has. Probiotics can also increase the number of beneficial bacteria by regulating the intestinal environment to improve the absorption rate of nutrients. At the same time, probiotics are also able to break down indigestible fibers and carbohydrates in the food so that they can be more easily absorbed and utilized by the body. This helps to reduce the incidence of indigestion and gastrointestinal discomfort. Dong Yuanyang et al. [42] observed the effect of adding *Enterococcus faecalis* and *Bacillus subtilis* to the diet of *Pigeons* on their growth performance. The results showed that compared with the control group, the fecal enterococci treatment group significantly increased the average daily weight gain from 12 to 28 days of age, and the two bacteria alone or in combination significantly increased the amylase activity in the duodenum, and also increased the abundance of segmented filamentous bacteria, *Bacillus cereus* and other strains of bacteria. Huang Jibing [43] studied the effects of different levels of compound probiotic preparations on the growth performance of weaned piglets, and the resuLts showed that the average daily feed intake of weaned piglets in test groups 1, 2 and 3 was higher than that of the control group, and the average daily weight gain of weaned piglets in test groups 2 and 3 was significantly higher than that of the control group, which indicated that the addition of a 2.0% compound probiotic preparation to the basal diet could improve the growth performance of crossbred weaned piglets.

(2) Enhance the body's immune system The intestine is an important part of the body's immune system, and probiotics can help enhance the barrier function of the intestine, prevent harmful substances from entering the body, and help the body to

resist the infringement of viruses and harmful bacteria, so as to improve the body's immune system [44-45].

(3) Maintain the balance of intestinal flora. When the intestinal flora is out of balance, it may lead to digestive problems and malabsorption of nutrients. Probiotics can compete with harmful bacteria in the body for living space, inhibit the growth of harmful bacteria, maintain the balance of intestinal flora, prevent diarrhea, constipation and other digestive problems.

(4) Reduction of intestinal inflammation Probiotics can reduce the occurrence of inflammatory reactions in the intestinal tract and reduce the impact of intestinal inflammation on the digestion and absorption of nutrients [46].

1.4.2 Introduction to *Lactic acid bacteria*

Lactic acid bacteria (LAB) are a unique group of non-spore-forming, Gram-positive bacteria that utilize fermentable carbohydrates as an energy source to produce large amounts of lactic acid. These bacteria are widely distributed in nature and have a rich diversity of species, including *Lactobacillus, Streptococcus, Bifidobacterium,* and *Schizococcus*, among other genera. Common species of *Lactobacillus* include Lactobacillus rhamnosus, *Lactobacillus acidophilus*, *Lactobacillus plantarum,* and *Lactobacillus teichii*. Most *Lactobacilli* are not motile, but a few species are able to move in a periplasmic motion. Their bodies are usually arranged in chains and have a unique morphology.

Lactobacilli live in a wide range of environments and are commonly found not only in foods and their products such as meat, milk, and vegetables, but also in the intestinal tracts of livestock and poultry, and even in some clinical samples. Especially in the oral cavity, intestinal tract and other environments of humans and other mammals, lactobacilli are key members of the normal microflora that make up a specific region. *Lactobacilli* play multiple roles in human health, and their main functions include facilitating the digestion and absorption of food, maintaining the balance and health of the intestinal flora, and enhancing the body’s immunity. In addition, as important microorganisms in fermented foods, *Lactobacilli* are an indispensable part of the daily diet, providing a wealth of choices for a healthy diet.

1.5 Introduction to *Scutellaria*

Scutellaria baicalensis Georgi is a traditional Chinese medicine in China, whose aliases are camellia root, earth gold tea root, etc. It belongs to the genus Scutellaria, family Labiatae, which is a perennial herbaceous plant. This plant has a fleshy rhizome that is plump, firm papery leaves that are lanceolate to linear-lanceolate, racemes that are terminal on the stem and branches, and corollas that are purple, purplish red to blue in color. *Scutellaria baicalensis* flowers between July and September, and grows mainly on sunny, grassy slopes at altitudes ranging from 60 to 1,300 (or 1,700 to 2,000) meters. *Scutellaria baicalensis* is used as a medicine with its roots, and the Compendium of Materia Medica records that it is bitter and cold in nature, and is attributed to the lung, gallbladder, spleen, large intestine, and small intestine meridians. *Scutellaria baicalensis* has various effects such as clearing heat and drying dampness, diarrhea and detoxification, stopping bleeding, and tranquilizing the fetus, etc. It is mainly used for treating warm-heat diseases, upper respiratory tract infections, coughing with lung-heat, jaundice with dampness-heat, pneumonia, dysentery, coughing up blood, redness of the eyes, restlessness of the fetus, hypertension, and carbuncles and boils.

The chemical composition of *Scutellaria baicalensis* mainly consists of flavonoids, polysaccharides, and volatile oils, etc., and among them, flavonoids including baicalein, baicalin, baicalein, hanhuangqin, and hanhuangqin, etc., are the most important medicinal components. These compounds give *Scutellaria baicalensis* a variety of pharmacological effects, such as.

(1) Bacteriostatic effect Scutellaria baicalensis exhibits a broader antimicrobial spectrum of activity, with good inhibitory effects on a variety of bacteria and fungi, including *Staphylococcus aureus*. Liu Baisuan et al. [47] obtained *Scutellaria baicalensis* extract by aqueous alcoholic precipitation method and showed through

their study that *Scutellaria baicalensis* extract had significant bacteriostatic activity against *S. aureus* with the lowest inhibitory concentration of 1.25 g/L. It was hypothesized that it might be possible that *Scutellaria baicalensis* extract caused the death of S. aureus by affecting the permeability of the cell membrane, which led to the leakage of macromolecules such as intracellular proteins, nucleic acids and other molecules. Xue Zhang [47] showed that 35 μg/mL baicalein-iron nanomaterials could completely kill Gram-negative bacteria, and there was a linear correlation between the concentration and time of the two, and the combination with H_2O_2 had excellent antibacterial effects on both Gram-negative and Gram-positive bacteria. It also showed that baicalein-iron nanomaterials have no obvious cytotoxicity and good hemocompatibility, and have good prospects for antibacterial applications.

(2) Antioxidant effects *Scutellaria baicalensis* is rich in flavonoid antioxidant substances, which can scavenge free radicals, delay aging, and protect cells from oxidative damage. Yang Shuaiyong et al. used [49] *Scutellaria baicalensis* flavonoid solution into the stomachs of mice, and the test results after 28 days showed that the serum total superoxide dismutase activity of mice was significantly or very significantly increased, and malondialdehyde level was significantly reduced, indicating that the antioxidant function of mice couLd be improved by gavage with *Scutellaria baicalensis* flavonoid. Chai Shouhong et al. [50] added different levels of baicalein to the diet of fattening pigs, and showed that the addition of baicalein at a high level (600 mg/kg) was able to increase the total antioxidant capacity (T-AOC) activity and antioxidant dismutase (SOD) activity of serum, and also increased the activities of immunoglobulins and interleukins in serum, thus improving the immune and antioxidant functions of pigs.

(3) Anti-inflammatory effects the flavonoids in *Scutellaria baicalensis* have obvious anti-inflammatory effects and can be used in the treatment of inflammatory diseases, such as dermatitis and intestinal inflammation. Hu Qing et al. [51] constructed an allergic rhinitis mouse model and set up several treatment groups, and the resuLts showed that the mucosal tissues of the baicalein-treated group gradually recovered, and

the data of all indexes decreased significantly. The resuLts showed that baicalein inhibited NF-κB p65, STAT3, and ERK protein phosphorylation, reduced inflammatory cell aggregation, attenuated inflammatory response, and alleviated allergic rhinitis in mice by regulating the NF-κB/STAT3/ERK signaling pathway.

(4) Other pharmacological effects such as anti-tumor, inhibition of cardiovascular and cerebrovascular diseases, and antidepressant. Some studies have shown that *Scutellaria baicalensis* also has some therapeutic effects on neo coronavirus pneumonia [52].

1.6 Applications of probiotic fermentation of *Scutellaria baicalensis*

Huang Haibin et al. [53] co-fermented compound Chinese herbs such as *Scutellaria baicalensis*, *Artemisia annua*, Sophora japonica, *and Glycyrrhiza glabra* with *Lactobacillus rhamnosus* and examined their therapeutic efficacy against chicken coccidiosis. The resuLts showed that the fermentation products significantly attenuated the pathological changes in the cecum and reduced the number of oocysts discharged from the infected chicks, which indicated that co-fermentation improved the therapeutic efficacy of the Chinese herbs for chicken coccidiosis.

Li Guozhong [54] used *Bacillus subtilis* to ferment *Scutellaria baicalensis*, determined the optimal fermentation conditions and applied them to pig feed, and tested growth performance, immune function and other indicators, which showed that the effect of adding fermented *Scutellaria baicalensis* group was better than that of the same additive amount of unfermented *Scutellaria baicalensis* group, and the overall effect was the best in the group with the addition of 0.15% fermented *Scutellaria baicalensis*. An Qi et al. [55] used *Lactobacillus plantarum* to re-ferment *Scutellaria baicalensis* dregs and add it to pig feed, and tested the indexes of weaned piglets, the resuLts showed that *Scutellaria baicalensis* dregs fermented by *Lactobacillus plantarum* as a feed additive could effectively increase the weight gain of piglets, reduce the rate of diarrhea of piglets, and help to improve the immune system of piglets, and regulate the microflora of piglets' intestinal tract.

Conclusions to chapter 1

Due to the high content of biologically active substances in the form of flavonoids, polysaccharides and alkaloids, medicinal plants (herbs) have long been used in Chinese medicine for the prevention and treatment of various diseases.

To improve the preventive and therapeutic effect of medicinal plants, they are usually subjected to extraction. Traditional extraction methods involve decoction, cold maceration and alcohol precipitation. The disadvantages of these methods include complexity, long duration and high levels of impurities. All this indicates ineffective use of the active ingredients of Chinese herbal medicines.

Compared with traditional herbal fermentation, improved technology and the use of modern equipment make it possible to reduce the influence of the subjective human factor, increase the efficiency and controllability of strain transformation, and obtain a higher and more stable quality of the finished product. Modern herbal fermentation uses strains that are engineered from purification of existing fermentation strains and improved through genetic means, cell engineering, etc. Fermentation with purification of both single and complex strains has been developed.

A review of the literature on modern technology for the fermentation of medicinal herbs and the influence of various factors on their pharmacological effectiveness was carried out. Information is provided about probiotics – a group of microorganisms that contribute to the normal function of the digestive system and strengthen the immune system. positive impact on a person's mental health. It has been established that the use of probiotics for the fermentation of herbal medicines helps to increase the content of active ingredients in the medicine and reduce toxic side effects.

A general understanding of *Lactic Acid Bacteria* is provided as a unique group of non-spore-forming Gram-positive bacteria that use fermentable carbohydrates as an energy source to produce large amounts of Lactic Acid. These bacteria are widespread in nature and have a rich diversity of species, including *Lactobacillus*, *Streptococcus*, *Bifidobacterium* and *Schizococcus*, as well as other genera. Common *Lactobacillus* species include *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus* *plantarum* and *Lactobacillus teichii*. *Lactobacilli* are important microorganisms in fermented foods and are an essential part of the daily diet, providing a wide range of healthy nutritional options.

A description is given of *Scutellaria baicalensis Georgi*, a widely used traditional Chinese medicine, the most important medicinal components of which are flavonoid and antioxidant substances that cause pharmacological, bacteriostatic, anti-inflammatory and antioxidant effects.

The results of modern studies showing the feasibility of probiotic fermentation of *Scutellaria baicalensis* are presented.

Based on the above, the purpose of the study is formulated – the Genome sequencing of existing lactic acid bacteria strains in the laboratory and selection of three strains for Scutellaria baicalensis fermentation, and to test the antioxidant and bacteriostatic indices of the fermentation broths by relevant methods.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE STUDY

The object and purpose of the study are given in the introduction; below is a description of the materials and methods of the study.

2.1 Content of the study

In this experiment, genome extraction and sequencing of existing laboratory strains were performed to identify three strains of *Lactobacillus* used in the fermentation of *Scutellaria baicalensis*, and the fermentation broth was tested for antioxidant and bacteriostatic indexes.

2.2 Experiment I: strain screening

2.2.1 Materials and Instruments

(1) Strain numbers: 7, 10, 82, H4, 58, 149, 2, 22, 8, 14, 110, 11842, H2. All are kept in Laboratory A217 in the Food Engineering Building.

(2) Instruments and equipment: autoclave sterilizer, electric blast drying oven, ultra-clean bench, centrifuge, pipette gun, PCR instrument, genome extraction kit (Nanjing Novozymes Bio-technology Co., Ltd.)

(3) Culture medium: MRS broth liquid medium

2.2.2 Test methods

(1) Strain activation: Weigh 14.79 g of MRS broth powder in a 500 mL beaker and add 300 mL of pure water, dispense into test tubes and sterilize at 115 °C for 30 min, cool and store at room temperature.

Using a pipette gun, the strain in the seed-preserving tube was resuspended by oscillation and 100 uL was aspirated and inoculated into MRS liquid medium for expansion, and incubated at 37 °C for 24 h.

(2) Extraction of genome: Sample processing:

1. Extract 1 mL of bacterial solution, centrifuge at 10000 rpm for 1 min, pour off the culture medium.

2. Add 180 uL Lysozyme, oscillate to make the bacteria resuspension, 37 ℃ water bath for 30min.

3. Add 20 uL *Proteinase K* and mix well with shaking.

4. Add 250 uL Buffer GB, shake and mix well, 70 ℃ water bath for 10min.

5. Add 4 uL *RNase A* to the digest, shake for 15 sec and leave for 5-15 min at room temperature.

Over-column purification:

1. Add 180 uL of anhydrous ethanol, shake and mix well, and briefly centrifuge to collect the liquid on the inner wall of the tube cap.

2. Transfer the mixture to a Fast Pure gDNA Mini Columns III adsorption column, which has been placed in a collection tube. centrifuge at 12,000 rpm for 1 min and discard the filtrate.

3. Add 500 uL Buffer PB to the adsorption column, centrifuge at 12000 rpm for 1min and discard the filtrate.

4. Add 600 uL Buffer PW to the adsorption column, centrifuge at 12000 rpm for 1min, discard the filtrate.

5. Repeat step 4.

6. Put the adsorption column back into the collection tube and centrifuge the empty tube at 12000 rpm for 2 min.

7. Transfer the adsorption column to a new centrifuge tube, open the lid to dry for 5 min, add 50 uL dd H_2O to the center of the adsorption column, leave it at room temperature for 5 min, and centrifuge at 12000 rpm for 1 min.

8. Discard the adsorption column and store the DNA product in a 4 °C refrigerator.

(3) Perform PCR amplification

1. Configure the PCR reaction system (Table 2.1).

Table 2.1 - **PCR reaction system**

Reagents	Volume, uL
Taq DNA polymerase	10
ddH_2O	7.5
Upstream primer 27F	1
Downstream primer 1492R	1
Samples	0.5

2. Configure the reaction system in the collection tube, put it into the PCR instrument for reaction, and set up the program for 16s genome amplification.

3. After the reaction, the samples were bagged and sent to Jinan Sangong Bioengineering Co. for 16 s gene sequencing.

(4) Seed preservation:

1. Extract 2 mL from each of the above MRS liquid medium and add it to a centrifuge tube, centrifuge at 6000 rpm for 3min, discard the supernatant.

2. Take 1 mL of fresh MRS liquid medium and add it to the above centrifuge tube, shake and resuspend, and mix the two centrifuge tubes and shake and resuspend.

3. Take the seed-preserving tube, add 1 mL of 50% glycerol, and then add the mixed bacterial solution from the centrifuge tube mentioned above to the seed-preserving tube and mark it well.

4. Put it into -20 °C refrigerator to chill.

2.3 Probiotic fermentation of *Scutellaria baicalensis*

2.3.1 Materials and Instruments

(1) Herbal ingredients: *Scutellaria baicalensis* 200 g.

(2) Instruments and equipment: autoclave sterilizer, electric blast drying oven, ultra-clean bench, centrifuge, pipette gun, pulverize, electronic weighing machine,

constant temperature incubation shaker, microwave oven, thermostatic water bath, blue-capped bottles.

(3) Bacterial strains: three strains were selected from the above preservation tubes, namely: 7 *Lactobacillus rhamnosus*, 22 *Lactobacillus plantarum*, 149 *Lactobacillus delbrueckii*, and *Micrococcus garciniae* (preservation of the seed in the laboratory of the Food and Engineering Building, A217).

(4) Culture medium: MRS liquid medium (49.3 g/1000 mL), S1 medium: tryptone 0.8%, Yeast extract 0.5%, dextrose 0.5%, sodium chloride 0.5%, disodium hydrogen phosphate 0.2%, agar powder 1.5% (agar powder was added directly to the conical flask).

2.3.2 Test methods

Grinding of herbal raw materials → autoclave sterilization and drying → probiotic strains → activation and transfer → inocuLation powder → shaking and homogenization → fermentation

(1) Scutellaria baicalensis pulverized and sterilized: Scutellaria baicalensis solids were powdered using a pulverize and stored in a -20 °C refrigerator. The sterilization method was referred to the study of Huang Long et al. [56] and improved. 60 g of *Scutellaria baicalensis* powder was taken and sterilized at 121 °C for 20 min and dried in an electric blast drying oven.

(2) Strain activation and transfer: Take 100uL each of *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus delbrueckii* and inoculate them in fresh MRS liquid medium, and incubate them at 37 ℃ for 24 h.

Take 100 mL of fresh MRS liquid medium, pour it into a blue-capped bottle and inoculate 10 mL of the above activated *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus desmosus* in an ultra-clean bench, and let it stand at 37 ℃ for 24 h.

Take 100 uL of *Micrococcus garciniae* and inoculate it in fresh S1 liquid medium, shake the bed at 37 °C and incubate it for 24 h before transferring.

(3) Inoculation of *Scutellaria baicalensis* powder: 10 g of *Scutellaria* powder was poured into a blue-capped bottle, labeled 7_1, 7_2, 22_1, 22_2, 149_1, and 149_2, and left to ferment at 37 C.

(4) Indicator testing: antioxidant and bacteriostatic indicators were selected for testing. The fermentation broth was extracted at different time days for DPPH scavenging rate, hydroxyl radical scavenging rate, and bacteriostatic test, respectively.

DPPH scavenging assay:

1. System configuration: DPPH: 0.0197 g/50 mL ethanol (10×), then diluted tenfold for use (1×); VC (ascorbic acid): 0.1 g/mL.

2. Sample addition: As: sample + DPPH = 1:3; Ac: sample + anhydrous ethanol = 1:3; Ab: water + DPPH = 1:3

3. Reaction: After adding the reagents and mixing, react for 30 min at room temperature, then centrifuge at 7500 rpm for 5 min and measure the absorbance at 517 nm.

$$\text{DPPH clearance rate} = 1 - \frac{\text{As}-\text{Ac}}{\text{Ab}} \times 100\% \qquad (2.1)$$

Hydroxyl radical scavenging system configuration:

1. PBS: 0.02 mol, pH = 7.4 (1000 mL pure water, 8.5 g sodium chloride, 2.2 g disodium hydrogen phosphate, 0.2 g sodium dihydrogen phosphate), o-diazophene: 0.0495 g/10 mL, $FeSO_4$ 0.007 g/10 mL, H_2O_2 100 uL/50mL pure water, VC (Ascorbic acid) 0.1 g/mL

2. Sample addition: ① PBS 1 mL + o-diazophene 0.5 mL + $FeSO_4$ 0.5 mL + H_2O_2 0.5 mL + fermentation broth 0.5 mL

② PBS 1 mL + o-diazophene 0.5 mL + $FeSO_4$ 0.5 mL + H_2O_2 0.5 mL + dd H_2O 0.5 mL

③ PBS 1 mL + o-diazophene 0.5 mL + $FeSO_4$ 0.5 mL + dd H_2O 1 mL

3. Reaction: 37 ℃ water bath reaction for 1 h, after 10000 rpm centrifugation for 5min, absorbance was measured at 536 nm.

$$\text{Hydroxyl radical clearance rate} = \frac{①-②}{③-②} \qquad (2.2)$$

Determination of the circle of inhibition: Oxford cup method was used to determine the circle of inhibition: 50 uL of Garcinia micrococcus bacterial liquid was aspirated in 100 mL S1 solid medium (at this time, the temperature of S1 solid medium was about 40 °C, not solidified), shaking and mixing well. Place 5 Oxford cups in the plate, spaced evenly. Perform an inversion of the plate. Allow the plate to solidify and then take out the Oxford cups. One 150 uL NiSin positive control, two Fermentation Solution No. 1 and two Fermentation Solution No. 2 were placed in the plate. The two plates were flatly placed in a 37 °C incubator for 24 h at constant temperature to observe whether the ring of inhibition appeared and the size of the ring of inhibition. Determination of the sensitivity of the inhibition circle: the diameter of the inhibition circle $d \leq 6$mm is insensitive, 6 mm $\leq d \leq$ 10 mm is low sensitivity, 10 mm $\leq d \leq$ 15 mm is medium sensitivity, 15 mm $\leq d \leq$ 20 mm is high sensitivity, $d \geq$ 20 mm high sensitivity.

Conclusions to chapter 2

The object and purpose of the study are stated in the introduction. Therefore, this chapter provides a description of the materials and research methods. Research methods are a way of acquiring reliable scientific knowledge, skills and practical knowledge in various fields of activity.

In this work, genome extraction and sequencing of existing laboratory strains were performed to identify three *Lactobacillus* strains used in the fermentation of *Scutellaria baicalensis*, and the fermentation broth was tested for antioxidant and bacteriostatic indices. With this in mind, the materials and methods that were used at each stage of the study are described.

CHAPTER 3
EXPERIMENTAL PART

3.1 Experiment I：Results of strain screening and sequencing

Table 3.1 − **Strain number and name**

Sample number	Strain name
7	*Lactobacillus rhamnosus*
10	
82	*Lactobacillus fermentum*
58	*Lactobacillus acidophilus*
2	*Lactobacillus plantarum*
22	
8	
14	
110	
11842	*Lactobacillus Germanus*
149	
H2	

3.2 Experiment II：Results of probiotic fermentation of *Scutellaria baicalensis*

1. DPPH clearance rate:

As can be seen from Table 3.2, with the increase of fermentation time, the DPPH clearance effect of *Lactobacillus rhamnosus* will gradually rise to a certain peak, and then begin to show a downward trend. With the increase of fermentation time 72 h before fermentation, the DPPH clearance effect was also enhanced, and the highest clearance rate was reached at 72 h. The fermentation of *Lactobacillus plantarum* and *Lactobacillus delleri* was carried out at 72 h.

Table 3.2 - **DPPH clearance rate of *Lactobacillus rhamnosus* No. 7 fermentation broth**

Fermentation time, h	DPPH clearance rate of fermentation broth, %	DPPH clearance rate of VC positive control, %
24	95.2%	97.9%
48	98.9%	
72	99.0%	
96	95.7%	

Table 3.3 - **DPPH clearance rates of *Lactobacillus plantarum* No. 22 fermentation broth, *Lactobacillus delleri* No. 149 fermentation broth**

Fermentation time, h	DPPH clearance rates, %		VC positive control DPPH clearance, %
	22	149	
24	85.8%	92.2%	97.4%
48	94.6%	93.3%	
72	95.4%	94.4%	

It can be seen from Table 3.2 and Table 3.3 that with the increase of fermentation time, the DPPH removal effect of *Lactobacillus plantarum* and *Lactobacillus* DPPH gradually increased. The DPPH removal effect of *Lactobacillus rhamnosus* fermentation broth was better than that of the other two strains.

2. Hydroxyl radical clearance rate:

As can be seen from Table 3.4, with the increase of fermentation time, the hydroxyl radical scavenging effect of *Lactobacillus rhamnosus* will gradually rise to a certain peak, and then begin to show a downward trend. With the increase of fermentation time 72 h before fermentation, the scavenging effect of hydroxyl radical was enhanced, and the highest clearance rate was reached at 72 h.

Table 3.4 - **Hydroxyl radical clearance rate of *Lactobacillus rhamnosus* No. 7 fermentation broth**

Fermentation time, h	Hydroxyl radical clearance rate, %	VC positive control, %
24	30.6%	84.7%
48	70.8%	
72	77.5%	
96	20.2%	

As can be seen from Table 3.4 and Table 3.5, the hydroxyl free radical scavenging effect of *Lactobacillus plantarum* and *Lactobacillus delleri* gradually increased with the increase of fermentation time. The scavenging effect of *Lactobacillus delleri* fermentation broth was better than that of the other two strain

Table 3.5 - **Hydroxyl radical clearance rate of Lactobacillus plantarum No. 22 fermentation broth, *Lactobacillus delleri* No. 149 fermentation broth**

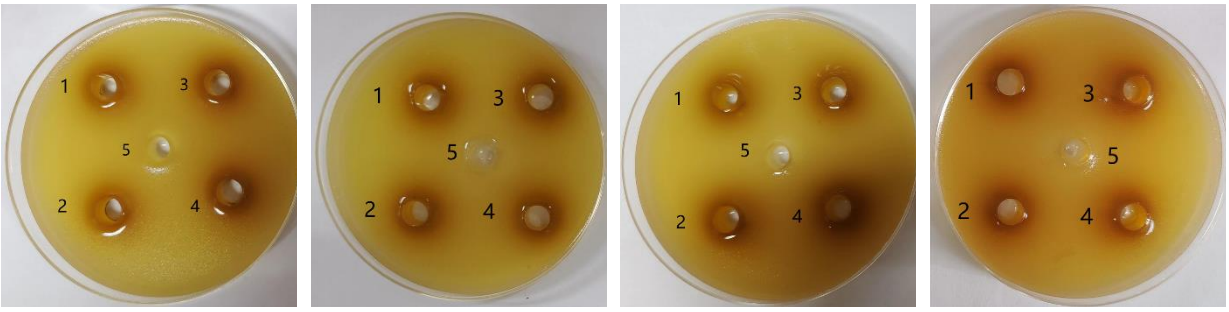
Fermentation time, h	Hydroxyl radical clearance rate, %		VC positive control, %
	22	149	98.7%
24	48.8%	31.5%	
48	61.7%	60.2%	
72	77.5%	84.9%	

3. Circle of inhibition size

As can be seen from Table 3.6 and Figure 3.1, with the increase of fermentation time, the antibacterial zone diameter of L. rhamnosus gradually rises to a certain peak value and then begins to show a downward trend. The diameter of the antibacterial zone expanded with the increase of fermentation time and reached the maximum diameter at 72 h before fermentation.

Table 3.6 - **Circle of inhibition size of *Lactobacillus rhamnosus* No. 7 fermentation broth**

Fermentation time, h	Diameter of inhibition circle of fermentation liquid, mm	Inhibition Circle Diameter of Nisin Positive Control, mm	Sensitivity
24	14	12.5	medium sensitivity
48	14.37		medium sensitivity
72	14.5		medium sensitivity
96	13.75		medium sensitivity

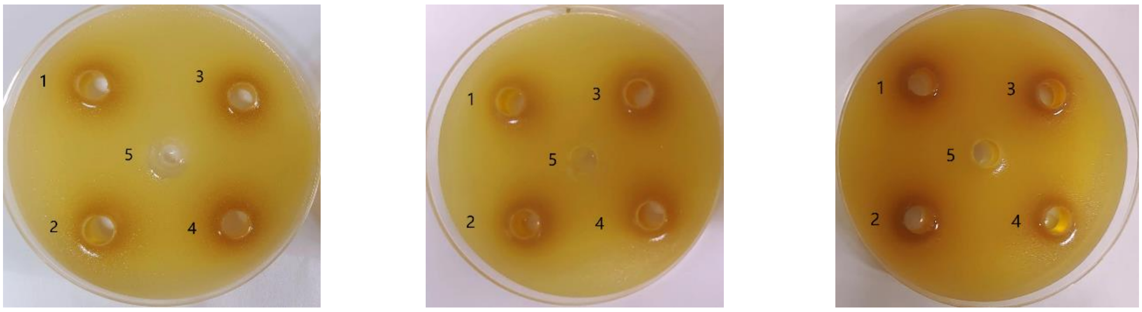


24 h 48 h 72 h 96 h

Figure 3.1 - **Inhibition circle diameter of *Lactobacillus rhamnosus* No. 7 fermentation broths with different fermentation times:** 1, 2 - 7_1 vials of fermentation broth; 3, 4 - 7_2 vials of fermentation broth; 5 - Nisin positive control

Table 3.7 - **Circle of inhibition size of *Lactobacillus plantarum* No. 22 fermentation broth, *Lactobacillus delleri* No. 149 fermentation broth**

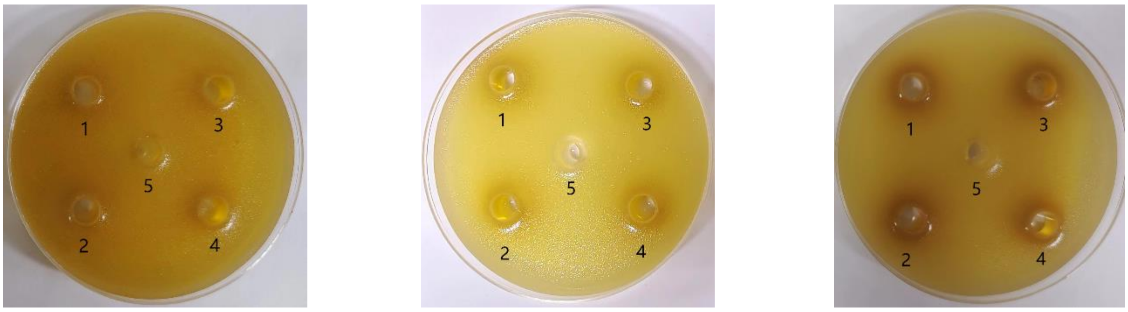
Fermentation time, h	Diameter of inhibition circle of fermentation liquid, mm		Inhibition Circle Diameter of Nisin Positive Control, mm	Sensitivity
	22	149		
24	13.4	12.3	12.5	medium sensitivity
48	13.5	13.5		medium sensitivity
72	13.75	14.1		medium sensitivity



24 h   48 h   72 h

Figure 3.2 - **Inhibition circle diameter of *Lactobacillus plantarum* No. 22 fermentation broths with different fermentation times:** 1, 2 - 22_1 vials of fermentation broth; 3, 4 - 22_2 vials of fermentation broth; 5 - Nisin positive control

As can be seen from Table 3.6, Table 3.7, Figure 3.2 and Figure 3.3, the diameter of antibacterial zone of *L. plantarum* and *L. delleri* gradually expands with the increase of fermentation time. The antibacterial effect of *Lactobacillus rhamnosus* fermentation broth was better than that of the other two strains.



24 h 48 h 72 h

Figure 3.3 - **Inhibition circle diameter of *Lactobacillus Desseri* No. 149 fermentation broths with different fermentation times:** 1,2 - 149_1 vials of fermentation broth; 3,4 - 149_2 vials of fermentation broth; 5 - Nisin positive control

Conclusions to chapter 3

Based on a review of the literature, it was found that thanks to modern fermentation technology, it is possible to effectively increase the content of active ingredients in medicinal herbs and obtain new beneficial ingredients, which opens up a new way for the further use of herbal medicine resources.

Probiotics are a class of microorganisms that can be used to increase the content of active ingredients in medications while reducing toxic side effects. *Scutellaria baicalensis*, a widely used Chinese herb in China, has pharmacological effects such as antioxidant, antibacterial and anti-inflammatory.

In this experiment, three strains of lactic acid bacteria were used to ferment Scutellaria baicalensis powder and tested for antioxidant and bacteriostatic properties. The results showed that *Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Lactobacillus delbrueckii* achieved maximum antioxidant and bacteriostatic indices measured after 72 hours of fermentation, with DPPH absorption: 99.02%, 95.4% and 94.5%, hydroxyl radical scavenging: 77 .5%, 77.5. % and 84.9%, and the diameter of the braking circle is 14.5 mm, 13.75 mm and 14.1 mm, respectively.

CONCLUSION

In this experiment, *Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Lactobacillus delbrueckii* were selected for the fermentation of scutellaria powder. The DPPH clearance rate, hydroxyl radical clearance rate and antibacterial index of antioxidant indexes were selected for testing. The results showed that the relevant indexes of fermentation liquid reached the best at 72 h fermentation, which were respectively: DPPH clearance rate: *Lactobacillus rhamnosus* 99.0%, *Lactobacillus plantarum* 95.4%, *Lactobacillus delleri* 94.4%; The clearance rate of hydroxyl free radical was 77.5% for Lactobacillus rhamnosus, 77.5% for *Lactobacillus plantarum* and 84.9% for *Lactobacillus delleri*. The diameter of inhibition zone was 14.5 mm for Lactobacillus rhamnosus, 13.8 mm for *Lactobacillus plantarum* and 14.1 mm for *Lactobacillus delleri*. In conclusion, the DPPH scavenging ability and antibacterial ability of *L. rhamnosus* were stronger than those of the other two strains, while the hydroxyl free radical scavenging ability of *L. rhamnosus* was stronger than those of the other two strains. It is speculated that *Lactobacillus rhamnosus* and *Lactobacillus delleri* have relatively complete and active antioxidant enzyme systems in the fermentation process, such as superoxide dismutase (SOD), catalase (CAT), etc., and more or more effective antibacterial active substances, such as lactic acid and antimicrobial peptides, may be produced in the fermentation process.

Thus, it showed stronger antioxidant capacity and antibacterial activity. Due to time constraints, the number of fermentation grams and the optimization of inoculated bacteria solution were not carried out in this experiment, so there is certain research space.

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