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 **Isolation of Lactic Acid Bacteria from Environmental Samples and Screening of Fermentation Capacity**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group
BEBT-21
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APPROVE
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«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Gao Tianli

1. Thesis topic **Isolation of Lactic Acid Bacteria from Environmental Samples and Screening of Fermentation Capacity**

Scientific supervisor Ph.D., Assoc. Prof. Olena Okhmat
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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

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WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
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10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)		

I am familiar with the task:

Student _____ Gao Tianli

Scientific supervisor _____ Olena OKHMAT

Abstract

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Lactic acid bacteria are a kind of probiotics widely distributed in nature. Because of their versatility and adaptability, lactic acid bacteria have attracted wide attention in many fields such as medicine, agriculture and industry. Lactic acid bacteria are widely found in various environments. Therefore, environmental samples such as water, soil and various plant surfaces are important sources of lactic acid bacteria. The purpose of this experiment was to isolate, identify and screen the fermentation ability of lactic acid bacteria in environmental samples. After the lactic acid bacteria in environmental samples were isolated and purified by plate streaking method, MRS solid medium was used to screen out the strains with soluble calcium ring or green color from environmental soil and environmental water sources. Finally, the screened strains were identified by morphological observation, physiological and biochemical tests and 16 S rRNA gene sequence alignment.

The species of lactic acid bacteria can be determined by comparing the 16 S rRNA sequence with the sequence in the database. The results showed that multiple strains of lactic acid bacteria were successfully screened from environmental samples. Strains X-1-1 and X-1-2 belong to *Lactobacillus plantarum*, strains X-4-1 and X-4-2 belong to *Lactobacillus brevis*, strains X-6-1 and X-6-2 belong to *Lactobacillus fermentum*, strains X-7-1 and X-7-2 belong to *Enterococcus faecalis*, strains X-10-1 and X-10-2 belong to *Lactobacillus paracasei*. The phylogenetic tree constructed by MEGA showed the genetic relationship and genetic differences between different strains. The results showed that strain X-1-1 and strain X-1-2 had the strongest genetic relationship, and the sequence alignment results showed that both strains belonged to *Lactobacillus*

plantarum. Through this study, the separation process, molecular identification method and screening process of lactic acid bacteria were systematically understood, and it was expected to provide ideas for the separation, screening and molecular identification of lactic acid bacteria.

Key words: Environmental samples, lactic acid bacteria, multiple sequence alignment, screening, molecular identification

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INTRODUCTION

Probiotics, especially lactic acid bacteria, can directly regulate the balance of intestinal flora and microbial environment. Therefore, probiotics and their metabolites can improve specific immune function. Due to its wide use as a starter in food production, it preserves and improves the flavor of various dairy products, meat, vegetables and cereal products, and lactic acid bacteria have a great market impact. As a functional food ingredient with potential health benefits, the market for lactic acid bacteria has also increased due to growing interest in probiotics. Lactic acid bacteria have many uses, including food and dairy industries, as probiotics to enhance human and animal health, as bio-fertilizers, and as a source of extracellular polysaccharides in various industries.

Lactic acid bacteria are a general term for a class of non-spore, Gram-positive bacteria, which play an important role in food fermentation. Lactic acid is mainly produced by fermentation of carbohydrates, which is generally considered to be safe. Lactic acid bacteria naturally exist in various environments, such as dairy products, meat, vegetables, human gastrointestinal tract, etc. In nature, lactic acid bacteria are widely found in various environments, including plants, human intestines and mucosa, as well as the surface of terrestrial and marine organisms.

Probiotics have health care functions such as preventing and alleviating diarrhea, regulating intestinal microflora, regulating immunity, and alleviating allergies. In addition, probiotics also help to treat a variety of diseases, such as gastrointestinal diseases, immune diseases and inflammatory bowel disease. It has also been reported that when probiotics are added to foods, they enhance the host 's immune system response, help digestion, and regulate the gastrointestinal microbiota. Lactic acid bacteria such as lactobacilli and enterococci are known as probiotics because they are thought to reduce diseases such as diarrhea, inflammatory bowel disease and irritable bowel syndrome.

Lactic acid bacteria are inexpensive, easy to cultivate, store and use, and easy to collect. Lactic acid bacteria are recognized as one of the most commonly used

microbial types in the world for the development of dairy and non-dairy fermented foods. Even now, this group of microorganisms is still one of the most studied microorganisms. Lactic acid bacteria have the potential to improve animal and crop productivity. In fruit trees, rice and horticultural crops, lactic acid bacteria strains are used as biocontrol bacteria and plant growth stimulants. Waste matrix waste from animals and mushrooms can be fermented and decomposed by lactic acid bacteria.

Because lactic acid bacteria have the ability to inhibit bacterial growth and promote plant growth, they can replace inorganic fertilizers and pesticides. Starch films were added by lactic acid bacteria to protect fruits and vegetables from oxidative damage. By using this strategy, the shelf life can be extended without compromising the established standards of the food packaging process. Lactic acid bacteria strains inhibit pathogenic microorganisms and improve animal health through nutrition, which makes it extremely beneficial to animal health. A large number of studies have shown that lactic acid bacteria can be isolated from feed, inhibit pathogenic pathogens, and support the gut microbiota of humans and animals. In addition, lactic acid bacteria are increasingly used commercially in the synthesis of chemicals, drugs and other beneficial commodities. Recent studies have shown that the synthesis of lactic acid by biotechnology can reduce the environmental pollution of the petrochemical industry. These new technologies will improve productivity and contribute to the sustainable development of agriculture and animal husbandry in the future.

CHAPTER 1
LITERATURE REVIEW

1.1 REVIEW OF LACTIC ACID BACTERIA

1.1.1 OVERVIEW OF LACTIC ACID BACTERIA

Lactic acid bacteria are a general term for a group of non-sporulating, Gram-positive bacteria that play an important role in food fermentation, mainly producing lactic acid by fermentation of carbohydrates, and are generally considered to be safe and without toxic side effects. Lactic acid bacteria are found naturally in various environments, such as dairy products, meat, vegetables, and human gastrointestinal tract. In nature, lactic acid bacteria are widely present in a variety of environments, including plants, the human gut and mucosa, and the surfaces of terrestrial and Marine organisms.

Due to the wide distribution of lactic acid bacteria in nature, it has high application value in important fields closely related to human life such as medicine, agriculture and industry, and has attracted great attention from people. It is widely used in the food and pharmaceutical industries for the production of fermented food and probiotics[1]. Currently, popular species such as *Diococcus, Pediococcus, Lactobacillus* , *Leuconococcus citritoticus, Lactococcus* and *Streptococcus* are widely used in these fields. These bacteria play a key role in processes ranging from food production to health promotion and environmental sustainability. Their versatility and adaptability make them an integral part of many aspects of human life and activities, and their importance is highlighted in many fields and industries around the world.

1.1.2 PHYSIOLOGICAL FUNCTIONS OF LACTIC ACID BACTERIA

It is generally believed that probiotics can regulate the structure of intestinal flora by increasing beneficial bacteria in the intestine and reducing pathogenic bacteria in the intestine to alleviate the disturbed intestinal flora and maintain the

health of the host[2]. Lactic acid bacteria form a diverse microbial clade known for their critical role in a variety of fermented foods and their significant prebiotic capabilities. These bacteria, mainly from *Lactobacillus* , *Bifidobacterium*, and other bacterial genera, provide a range of health advantages when integrated into the diet, mainly due to their probiotic functions. The core of the probiotic effect of lactic acid bacteria is its ability to benefit the host gastrointestinal system. Lactic acid bacteria inhibit the growth of harmful bacteria through mechanisms such as competitive exclusion and the production of antimicrobial substances such as hydrogen peroxide, organic acids, and bacteriocins, which contribute to the establishment of a balanced gut microbiota. By regulating the composition of intestinal microbes, lactic acid bacteria play an important role in maintaining and optimizing intestinal function. Their presence creates an environment conducive to digestive health and overall welfare, highlighting their importance in promoting gut homeostasis and microbes

Another important probiotic function of lactic acid bacteria involves a major effect on the immune system. These bacteria bind to gut-associated lymphoid tissues (GALT), prompting the production and regulation of many compounds, such as cytokines and immunoglobulins. This interaction plays an important role in regulating the immune system at the effector site to foster tolerance to innocuous antigens while enhancing defense against pathogens. Fermented foods are rich in lactic acid bacteria, which can produce organic acids, ethanol and antibacterial substances, and effectively inhibit spoilage bacteria and pathogenic bacteria in fermented foods. In addition, lactic acid bacteria, as key microorganisms in probiotics, occupy an important position in the intestinal microbiota of humans and mammals and exert important health promoting effects on the host. Numerous studies have shown that fermented foods, such as kimchi and sauerkraut[3], can enhance the immune system and reduce inflammation. Evidence supporting the important health-promoting and immunomodulatory properties of probiotics is also growing.

The metabolic regulation of lactic acid bacteria is one of its important physiological functions, involving energy balance, lipid metabolism, glucose metabolism and vitamin synthesis. Metabolites secreted by lactic acid bacteria can inhibit the activity of intestinal α-glucosidase and delay the breakdown of carbohydrates and glucose absorption. By stimulating intestinal L-cells to secrete glucagon-like peptide-1 (GLP-1), lactic acid bacteria can promote islet β cell proliferation and enhance insulin secretion, thereby improving glucose metabolism and insulin sensitivity. Lactic acid bacteria also play an important role in the regulation of uric acid metabolism, which has broad application prospects in the prevention and treatment of hyperuricemia and its related diseases[4.]

As an important metabolic by-product in the tumor microenvironment[5], lactate has attracted extensive attention for its role in the immune escape mechanism. Tumor cells produce and release a large amount of lactate into the tumor microenvironment through abnormal glycolysis (Warburg effect), resulting in a decrease in ph value. Elevated lactate levels seriously inhibit the proliferation, cytotoxic function and migration ability of immune effector cells such as macrophages and natural killer cells at the tumor site. In addition, lactate can regulate the expression of surface molecules of immune cells, interfere with their recognition and attack on tumor cells, and regulate signaling pathways to promote the expansion and enhancement of immunosuppressive cells such as regulatory T cells, thereby promoting immune tolerance in the tumor microenvironment. These approaches, when combined with existing immunotherapies such as immune checkpoint inhibitors and chimeric antigen receptor T-cell therapies, are expected to enhance the ability of the immune system to eliminate tumor cells. This could pave the way for novel combination therapy strategies in clinical cancer treatment to effectively overcome the phenomenon of tumor immune escape and ultimately improve the overall therapeutic effect.

1.2 ISOLATION OF LACTIC ACID BACTERIA AND SCREENING OF FERMENTATION CAPACITY

1.2.1 METHODS FOR ISOLATION OF LACTIC ACID BACTERIA

There are various methods for the isolation of lactic acid bacteria, such as pouring plate method, plate marking method, coating plate method and density gradient centrifugation[6].

Pour Plate Method is a common technique for the isolation and counting of microorganisms. Samples are mixed with melted AGAR medium and poured into sterile Petri dishes, which are incubated after solidification, and finally dispersed colonies are formed. This method is suitable for the isolation of aerobes, facultative anaerobes, and microaerobes, especially when the concentration of microorganisms in the sample is high.

Streak Plate Method is the most classical isolation and purification technique, in which inoculation rings are continuously streaking on the plate to gradually reduce the microbial density in the sample, and finally achieve the dispersion of individual microorganisms. Each independent colony is theoretically propagated from a single microbial cell to ensure genetic uniformity. Plate scribe method can inhibit the growth of high concentration of miscellaneous bacteria by dividing lines, and can effectively improve the separation efficiency of target bacteria.

Spread Plate Method is suitable for the isolation of aerobic and facultative anaerobes. Its core is to uniformly coat the bacterial solution on the AGAR surface by gradient dilution of the sample to form dispersed single colonies. The coated plate method is easy to operate, does not require melting medium, and is suitable for prefabricated AGAR plates. The colonies were only distributed on the surface, which was easy to observe the morphology and subsequent picking, and was friendly to heat-sensitive bacteria without high temperature exposure, which was conducive to protecting the activity of vulnerable microorganisms. However, aerobic bacteria need to be strictly limited, and improper operation can easily lead

to accumulation or uneven distribution of bacteria solution, and inaccurate dilution gradient will directly affect the counting results.

MRS Medium is a universal bacterial culture medium, which is rich in nutrients and suitable for the cultivation of various bacteria. MC medium can be used to distinguish lactose-producing bacteria from lactose-non-producing bacteria due to its special composition. Lactose-producing bacteria in microorganisms are more than lactic acid bacteria, so MRS Solid medium is used for the isolation of lactic acid bacteria[7].

1.2.2 IDENTIFICATION METHOD OF LACTIC ACID BACTERIA

Phenotypic characterization method : Lactic acid bacteria are classified according to their morphology or surface receptor specificity, such as morphological observation, physiological and biochemical characteristics monitoring and chemical classification characteristics. The phenotypic characteristics method is low in cost and can directly observe the morphology and metabolic characteristics of the bacteria. The phenotypic characteristics are directly related to the practical application of the strain. However, the phenotypic characteristics method takes a long time, and the scope of application and identification are limited. It is difficult to distinguish closely related species with similar phenotypes, and it is susceptible to environmental factors.

DNA marker technology is a kind of molecular biological identification method based on DNA sequence polymorphism for microbial identification, typing and genetic analysis. It mainly includes the extraction of bacterial genomic DNA, 16 S rDNA specific primer PCR amplification[8], purification of amplification products, DNA sequencing and sequence alignment.

The identification of lactic acid bacteria needs to be carried out in a variety of ways to ensure the accuracy of the identification results.

1.3 PHYLOGENETIC TREE

Phylogenetic tree is a tree diagram used to represent the evolutionary relationship between species, genes or other biological entities in biology. It shows the genetic relationship of different biological groups in the evolutionary history through branch structure and node relationship.

The phylogenetic tree[9] is a phylogenetic relationship between biological groups based on biological information, and infers the order of differentiation between biological groups from the last common ancestor. Obtaining the results of phylogenetic analysis is crucial because it can help reveal the evolutionary relationship between biological groups.

1.4 RESEARCH BACKGROUND AND CURRENT SITUATION AT HOME AND ABROAD

In recent years, significant progress has been made in the research of lactic acid bacteria. Internationally, lactic acid biohydrogen production technology has emerged[10], which uses food waste as a substrate to evaluate pretreated sludge and local microbial communities as inoculums. During the fermentation process, HLa and propionic acid were mainly converted to butyric acid and acetic acid. This study demonstrated the feasibility of using different inoculums to obtain H2 from complex substrates.

The combined application of *Lactobacillus* probiotics enhances the anti-tumor effect of telmisartan in rats. Recent studies have shown that the regulation of intestinal microbiome can obtain therapeutic benefits. The unique combination therapy using *Lactobacillus* mixture and telmisartan effectively reduced the levels of VEGF and IL-6, indicating a reduction in angiogenesis and inflammation. Combined administration of lactobacilli and telmisartan promoted programmed cell death, reversed disorders, improved histopathological results, and reduced carcinoembryonic antigen levels. These findings provide a new

perspective for the role of *Lactobacillus* and telmisartan in the treatment of colorectal cancer[11]

Chinese traditional application foundation is deep, the use of lactic acid bacteria has a long history, such as yogurt, pickles, soy sauce and other traditional fermented food production. However, modern scientific research started late. After the 1990 s, with the rise of the concept of probiotics, research has gradually accelerated.

In China, some research results have revealed that the combination of Rosa roxburghii Tratt[12] with *Pediococcus acidilactici* and *Lactobacillus plantarum* has synergistic effects of preventing drunkenness and promoting awakening, promoting ethanol metabolism, and protecting acute drunkenness-induced liver injury. It has laid an important foundation for further improving the utilization of probiotics and Rosa roxburghii Tratt resources and developing related functional products.

The application of lactic acid bacteria in the growth of aquatic animals[13] has been progressed. Lactic acid bacteria can improve the growth performance of aquatic animals, improve their intestinal health, and enhance their disease resistance. It has the advantages of no pollution, good effect, and no side effects. Lactic acid bacteria as a green additive is the trend of future development.

In summary, lactic acid bacteria research is moving from traditional applications to a new stage of precision and engineering. It is necessary to break through the bottleneck of strain resources and technology in China and strengthen clinical verification. The international frontier focuses on synthetic biology and cross-domain innovation. In the future, lactic acid bacteria may become the core tool of ' cell factory ' and personalized medicine, and will shine in aquaculture and industrial and agricultural production, but its development needs to balance technological innovation and safety supervision.

Conclusions to chapter 1

1. Overview of lactic acid bacteria: Definition and distribution: Lactic acid bacteria are a class of Gram-positive, spore-free bacteria, which are widely found in dairy products, human intestine, plant surfaces and other natural environments. Common genera include *Lactobacillus*, *Bifidobacterium*.

Application value: Because of its safety and lactic acid production characteristics, it is widely used in food fermentation (such as yogurt, pickles), medicine (probiotics) and industrial fields, and has the potential to promote health and environmental sustainable development.

2. Physiological function: Regulating intestinal flora : maintaining intestinal microecological balance and improving digestive health by competitively inhibiting harmful bacteria and secreting antibacterial substances (such as bacteriocins and organic acids); Immune regulation : Stimulate intestinal-associated lymphoid tissue (GALT), promote the secretion of cytokines and immunoglobulins, enhance anti-pathogen ability, and reduce inflammation (such as the role of fermented foods such as pickles); Metabolic regulation : improve glucose metabolism (inhibit α-glucosidase, promote insulin secretion), regulate lipid metabolism, and play a potential role in the prevention and treatment of uric acid metabolism and hyperuricemia;Anti-tumor potential : By inhibiting lactic acid-mediated immunosuppression in the tumor microenvironment (such as reducing NK cell toxicity), combined with immunotherapy (such as CAR-T cells), the anti-tumor effect is enhanced.

3. Separation and screening techniques : Separation methods : pouring plate method, plate line method, coating plate method, commonly used media include MRS (general) and MC (lactose identification) ; identification method : phenotypic method : morphological observation, biochemical characteristics (low cost but time-consuming, limited accuracy) ; molecular techniques : 16S rDNA sequencing (high-precision, distinguishing closely related species).

4. Phylogenetic tree: used to analyze the evolutionary relationship of lactic acid bacteria, the branch structure was constructed by gene sequence (such as 16S rRNA), and the common ancestor and differentiation order of different genera were revealed.

5. Research status and trends: International frontier: lactic acid biological hydrogen production technology (using food waste to produce hydrogen) ; synthetic biology and engineering applications (such as lactic acid bacteria combined with drugs to enhance anti-tumor effects). Domestic progress: research on traditional fermented foods (such as the hepatoprotective and hangover function of *R.roxburghii* compound probiotics); aquaculture applications (improving intestinal health, replacing antibiotics).

6. As a multifunctional microorganism, lactic acid bacteria play an irreplaceable role in health, food, environment and other fields. Future research will focus on genetic engineering optimization, clinical validation and interdisciplinary applications, promoting its transformation from traditional fermentation to ' cell factory ' and precision medicine.

CHAPTER 2

OBJECT, PURPOSE, AND METHODS OF THE STUDY

Preserved strains were screened for the laboratory. MRS liquid medium, nutrient agar, PCR reagents, bacterial genomic DNA extraction kit, trypsin, pepsin, yeast powder.

Research Insights – Provide insights into LAB isolation, screening, and identification processes for future studies. Object of study – lactic acid bacteria. Subject of study – Environmental sample isolation

2.1 EXPERIMENTAL INSTRUMENTS

Table 2.1 – Experimental instruments

Name of instrument	Manufacturers
Centrifuge	Eppendorf, Germany
High pressure steam sterilization pot	Jiangsu Xundi Instrument Technology Co., Ltd.
PCR Amplifier	Shandong Hengmei Electronic Technology Co., Ltd.
Ultra-clean worktable	Suzhou Antai Air Technology Co., Ltd.
Gel electrophoresis instrument	Beijing Liuyi Instrument Factory
Gel imaging system	Shandong Holder Electronic Technology Co., Ltd.
Electronic balances	Shanghai Mettler-Toledo Instrument Co., Ltd.
Magnetic stirrer	Shanghai Si Le Instrument Co., Ltd.
Biochemical incubator	Guangdong Medical Device Factory

2.2 EXPERIMENTAL DESIGN

2.2.1 MEDIUM CONFIGURATION AND SEPARATION TECHNOLOGY

MRS medium contained peptone, beef extract, glucose and $CaCO_3$, which was suitable for the isolation of *Lactobacillus*, and $CaCO_3$ dissolution circle was used to assist the screening of acid-producing bacteria. The improved

CHALMERS medium has a higher detection rate and is suitable for the separation of *Lactococcus*. The BCP medium is a bromocresol purple indicator, and the acid-producing colonies are yellow around, which is convenient for rapid identification.

The MRS solid medium was prepared as follows: 10.0 g peptone, 10.0 g yeast extract powder, 2.0 g potassium dihydrogen phosphate, 20.0 g glucose, 1.0 mL Tween 80, 0.1 g magnesium chloride, 0.2 g magnesium sulfate, 8 g beef powder, 5.0 g sodium acetate, 2.0 g diammonium hydrogen citrate, 0.05 g manganese sulfate, 15.0 g agar powder and 1000 mL distilled water were weighed with a balance. Each component was added to a large beaker and mixed evenly on a magnetic stirrer to ensure that the pH value was 5.7 ± 0.2. After mixing evenly, it was equally divided into several triangular flasks. Sealed and placed in a 121 ° C autoclave heated to 121 ° C high temperature sterilization for 20 minutes.

The method of dissolving calcium circle is that lactic acid dissolves calcium carbonate to form a transparent circle in a medium containing $CaCO_3$, and a single colony with a large transparent circle and clear colony edge is preferentially selected. The coating method and the streaking method are the inverted culture after the diluent is coated, or the strains are purified by multiple streaks to ensure the separation of single colonies.

After the sterilization was completed, the medium was taken out from the high-pressure steam sterilization pot, and the solid medium was placed in a microwave oven to melt. After it was completely melted, it was poured into a petri dish and waited for cooling.

A total of 10 g samples were mixed with 90 mL normal saline, and the mixture was diluted with sterile normal saline. The dilutions of 10-4,10-5 and 10-6 were taken, and the dilutions were moved into a cooled petri dish with a pipette gun in an ultra-clean bench. The diluted solution was evenly coated in a MRS solid petri dish containing CaCO3 or bromomethylphenol green using a burnt and sterilized cooled coating device or a disposable coating device, and coated in turn

from low concentration to high concentration. After the coating was completed, the petri dish was placed at 37 °C for 48 h.

The colonies with obvious calcium-dissolving ring or green color were selected for plate streaking on MRS culture dish, and the streaking was repeated many times until the pure single colony was obtained.

2.2.2 STRAIN IDENTIFICATION AND FUNCTIONAL EVALUATION OF LACTIC ACID BACTERIA

Gram staining (positive), cell morphology and arrangement were observed under microscope. Catalase reaction (negative), sugar fermentation test (lactic acid production) and nitrate reduction test were carried out by biochemical test.

The isolated pure strains were inoculated into MRS solid culture dishes and cultured at 37 ° C for 48 h. After the cultivation was completed, a single colony with smooth surface and no mixed impurities was selected, and two purification operations were repeated to observe the morphological characteristics of the colony in detail. Then, the inoculation ring was used to accurately pick out the colonies with good growth conditions, and smear them. After cooling and stabilizing them, Gram staining was performed. The morphological structure of lactic acid bacteria cells was carefully observed under the microscope, and the significantly positive was lactic acid bacteria. This procedure is not only beneficial to determine the purity of the strain, but also provides an important reference for the identification and subsequent use of the strain.

Through the determination of catalase, the medium used for the determination of catalase was PGY medium. Firstly, the isolated strains were inoculated on the slope of PGY medium and cultured at 37 °C for 24 hours. Then, an appropriate amount of inoculum was taken from the inclined surface of the culture medium and evenly spread on the surface of the clean glass slide. Subsequently, 5 % hydrogen peroxide solution was added to the glass slide coated with colonies, and the formation of bubbles was carefully observed. If bubbles were observed on the slide, it was judged to be catalase positive reaction ; if there is no bubble formation, it is

judged to be a negative reaction. After completing the above operation, the experimental results were recorded in detail, and the strains with negative reaction were screened.

The species were identified by PCR amplification and sequence alignment using 16 S rRNA sequencing.

(1) DNA extraction : The bacterial liquid of the isolated strain was cultured overnight and placed in a centrifuge tube. The centrifuge tube was placed in a centrifuge at 12000 rpm for 30 seconds, the supernatant was discarded, and the bacteria were collected.

The STE buffer solution containing lysozyme was added to the precipitate to re-suspend to destroy the cell wall and cell membrane of the bacteria and release DNA.

Then, an equal volume of phenol: chloroform : isoamyl alcohol mixture was added to the centrifuge tube to make it mixed evenly, centrifuged, and repeated this step many times.

Add an appropriate amount of isopropanol to the supernatant to precipitate DNA. The precipitate was washed with anhydrous ethanol, and the adsorption column was taken out in a clean centrifuge tube. The elution buffer was added, placed at room temperature for 2 minutes, and centrifuged at 12,000 rpm for 1 minute.

(2) PCR amplification : The 16S rRNA gene fragment was obtained by PCR amplification of the previously isolated strain DNA. The primer sequences of PCR amplification were 27F (5 ' -CAGAGTTTGATCCTGGCTCAG-3 ') and 1492R (5 ' -GGTTACCTTGTTACGACTT-3 '). The reaction system (25 μL) was as follows : 1 μL primer 27F and 1 μL primer 1492R (10 μmol · L − 1), 12.5 μL 2 × Master Mix, and ddH2O was supplemented to 25 μL. Fresh colonies cultured for 24-48 h were picked and mixed in the reaction system. The PCR reaction program was first pre-denaturation at 95 °C for 5 minutes, followed by 35 cycles, each cycle including : denaturation at 95 °C for 15 seconds, annealing at 65 °C for 30 seconds, and extension at 72 °C for 30 seconds. After the reaction was completed, it was

kept at 72 °C for another 7 minutes to ensure the complete extension of the product.

(3) Electrophoresis of PCR amplification products : The PCR products were purified and verified by gel electrophoresis to ensure that the amplified sequence was the 16S rRNA gene of lactic acid bacteria. The PCR amplification product was mixed with the supernatant sample buffer, and electrophoresis with DNA Marker at 1 % agarose gel 60 V for 20 min, and the results were observed by gel imager.

(4) Sequencing and sequence alignment : PCR amplification products were purified and sent to the sequencing company for sequencing. The sequencing results were submitted to the NCBI website for sequence alignment with the sequences in the database to determine the classification of each strain. By comparing the sequencing results with the 16S rRNA gene sequence database of known lactic acid bacteria, the species information of the isolated lactic acid bacteria can be identified.

(5) Construction of phylogenetic tree : The 16S rDNA sequences of 10 successfully sequenced lactic acid bacteria strains from different species were selected, and ClustalW multi-sequence alignment was performed using MEGA 11 software to analyze the differences and construct a phylogenetic tree.

The acid-producing ability was tested, and the pH change of fermentation broth was determined. The adaptability of the strain to acid (pH 3.0-4.0), salt (6 % NaCl) and temperature was evaluated by tolerance test.

Conclusions to chapter 2

1. Experimental materials. Strains: laboratory screening and preservation of strains ; medium and reagents

2. Experimental instruments and reagents. Main instruments: Centrifuge Eppendorf, Germany; high pressure steam sterilization pot Jiangsu Xundi Instrument Technology Co., Ltd.; pCR amplification instrument Shandong Hengmei Electronic Technology Co., Ltd.; gel electrophoresis apparatus Beijing Liuyi Instrument Factory; biochemical incubator Guangdong Medical Device Factory. Other equipment : triangular flasks, petri dishes, pipette guns, centrifuge tubes (10 ml, 2 ml), EP tubes, etc.

3. Medium configuration and separation technology. Selective medium : MRS medium : containing peptone, beef extract, glucose and CaCO3 (auxiliary screening acid-producing bacteria, dissolution circle observation). Improved CHALMERS medium : special for separation of Lactococcus. BCP medium : containing bromocresol purple indicator, acid-producing colonies showed yellow. MRS solid medium configuration : Components : peptone (10.0 g), glucose (20.0 g), agar (15.0 g), etc. (a total of 12 components).

4. Morphological and biochemical identification. Morphological observation: Gram staining (positive), microscopic observation of bacterial morphology (rod or spherical). Biochemical test : catalase test : negative (no bubble formation). Sugar fermentation test : verification of lactic acid production ability. Nitrate reduction test : Detection of metabolic characteristics. Molecular biology identification : DNA extraction : lysozyme wall-breaking → phenol-chloroform purification → isopropanol precipitation DNA. PCR amplification : Primers : 27F (5 ′ -CAGAGTTTGATCCTGGCTCAG-3 ′) and 1492R (5 ′ -GGTTACCTTGTTACGACTT-3 ′).

CHAPTER 3

EXPERIMENTAL PART

3.1 SEQUENCING RESULTS OF PCR AMPLIFICATION PRODUCTS

The PCR amplification product was sequenced, and the sequence fragment of X-1-1 was obtained after sequencing:

GGTGCTATACATGCAGTCGAACGAACTCTGGTATTGATTGGTGCTT
GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGT
GGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTA
ATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCT
TCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGG
GTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAAT
CGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGC
AGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGC
GTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGA
ACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA
GCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAA
GTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACT
GGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGT
GAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCT
GGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGAT
TAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGG
AGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCT
GGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAAC
CTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCC,

The sequencing map fragments are shown in Fig.3.1.

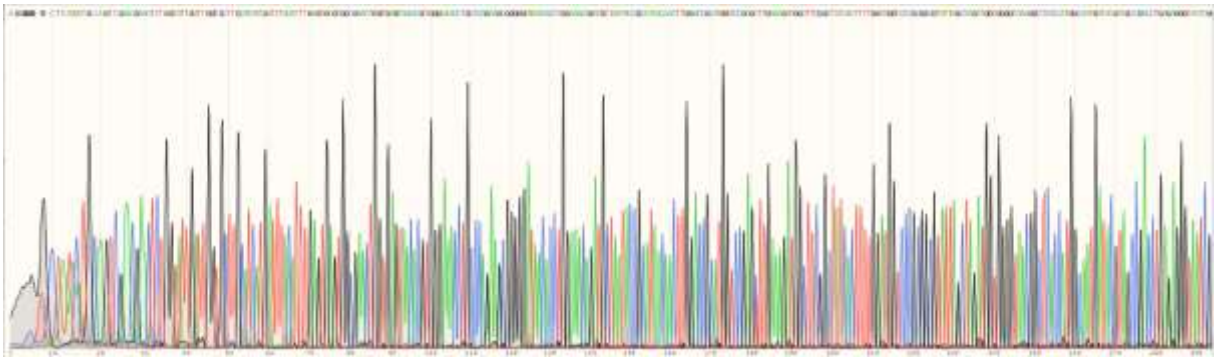


Figure 3.1 – X-1-1 sequencing map fragment

The X-1-2 sequence fragment is :

GGGGGGTGCTATACATGCAGTCGAACGAACTCTGGTATTGATTGGT
GCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACA
CGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATG
CTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATG
GCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGT
GGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGT
AATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGC
AGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGC
CGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGA
AGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAG
AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTT
TAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAA
ACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGC
GGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTG
TCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAG
GATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTT
GGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGC
CTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGA

ACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTC
CCTTC, The sequencing map fragments are shown in Fig.3.2.

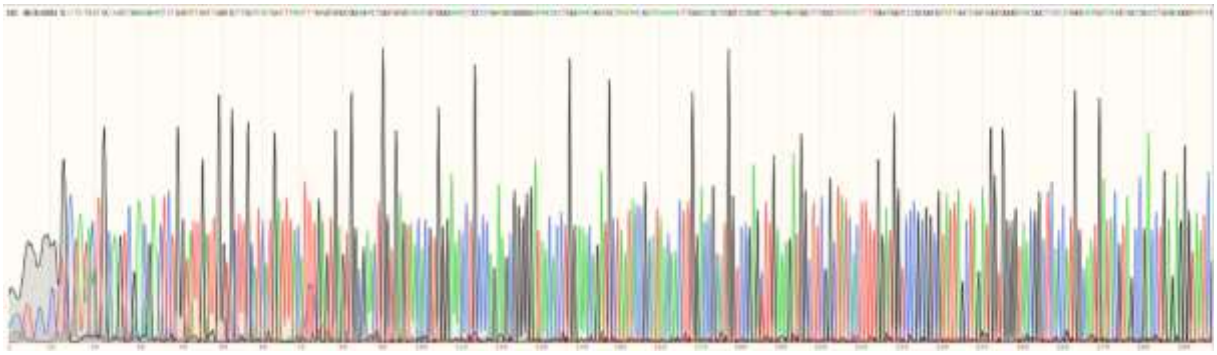


Figure 3.2 – X-1-2 sequencing map fragment

The sequence fragment of X-4-1 is:

GGTGGCGGCTGCTATACATGCAAGTCGAACGAGCTTCCGTTGAAT
GACGTGCTTGCACTGATTTCAACAATGAAGCGAGTGGCGAACTGGTGA
GTAACACGTGGGAAATCTGCCCAGAAGCAGGGGATAACACTTGGAAAC
AGGTGCTAATACCGTATAACAACAAAATCCGCATGGATTTTGTTTGAAA
GGTGGCTTCGGCTATCACTTCTGGATGATCCCGCGGCGTATTAGTTAGT
TGGTGAGGTAAAGGCCCACCAAGACGATGATACGTAGCCGACCTGAGA
GGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCA
ATGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAA
AGAAGAACACCTTTGAGAGTAACTGTTCAAGGGTTGACGGTATTTAAC
CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGT
TTTTTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTGCATCG
GAAACTGGGAGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGT
AGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCG
GCTGTCTAGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGA
ACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAA
GTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCAC
TCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACG

GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCG
AAGAACCTTACCAGGTCTTGACATCTTCTGCCAATCTTAGAGATAAGAC
GTTCCCTTCGGGGACAGAATGACAGGTGGTGCATGGTTGTCGTCAGCTC
GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTA
TCAGTTGCCAGCATTCAGTTGGGCACTCTGGTGAGACTGCCGGTGACAA
ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACC
TGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCGAAGTCG
TGAGGCTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGATTGTAGGCT
GCAACTCGCCTACATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCA
TGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC
ATGAGAGTTTGTAACACCCAAAGCCGGTGAGATAACCTTCGGGAGTCA
GCCGTCTAACGGTGATAT, The sequencing map fragments are shown in Fig.3.3.

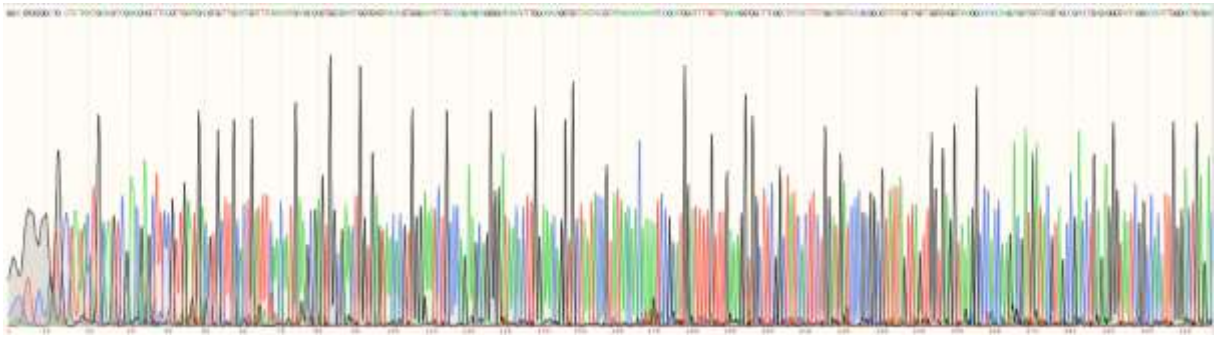


Figure 3.3 – X-4-1 sequencing fragment

The sequencing fragments of strains X-4-2, X-6-1, X-6-2, X-7-1, X-7-2, X-10-1 and X-10-2 are shown in Fig. 3.4.

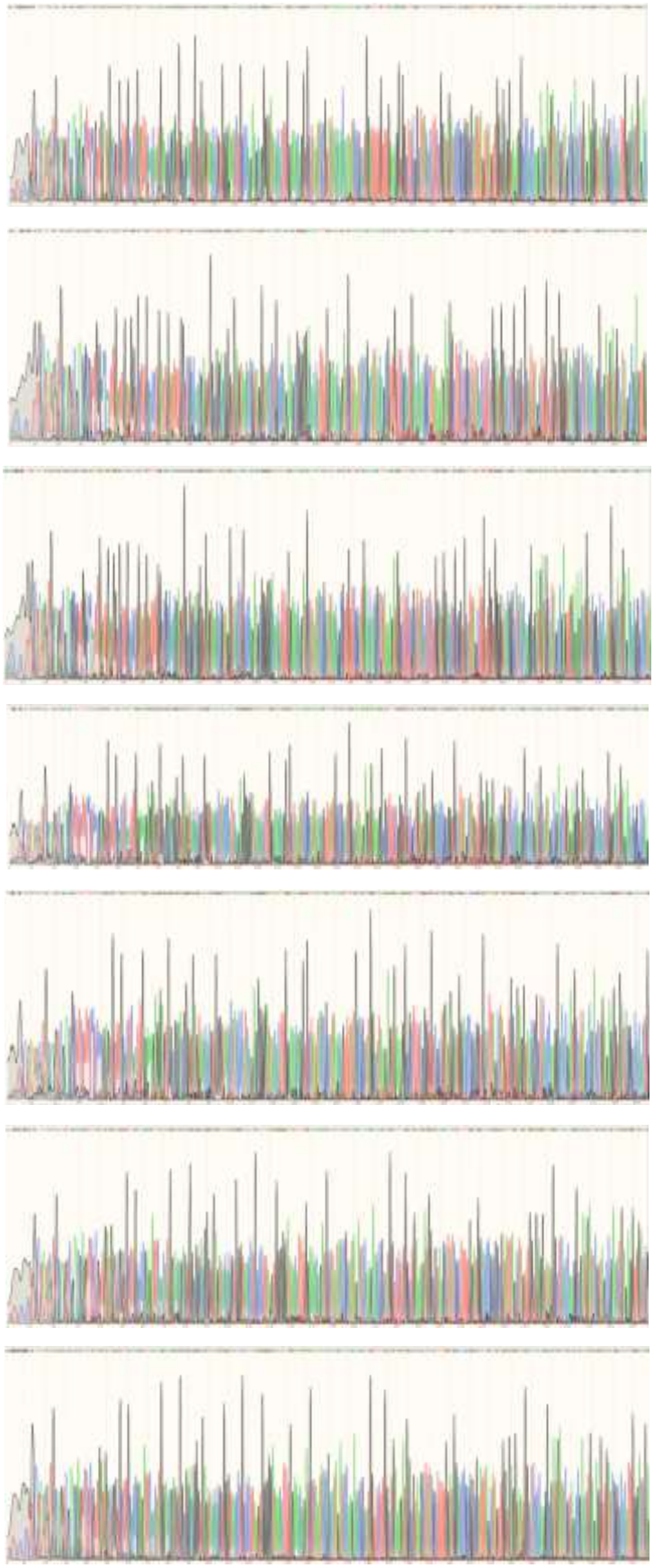


Figure 3.4 – From top to bottom are X-4-2, X-6-1, X-6-2, X-7-1, X-7-2, X-10-1, X-10-2 sequencing map fragments.

3.2 SEQUENCE COMPARISON RESULTS

Enter the NCBI official website, enter the sequence to be aligned of X-1-1, perform BLAST, and compare the sequence to be aligned with the sequence in the database. The alignment results show that the strain with the highest similarity to X-1-1 is *Lactobacillus plantarum* (Fig. 3.5). The Query Cover value is 100 %, and X-1-1 is determined as *Lactobacillus plantarum*.

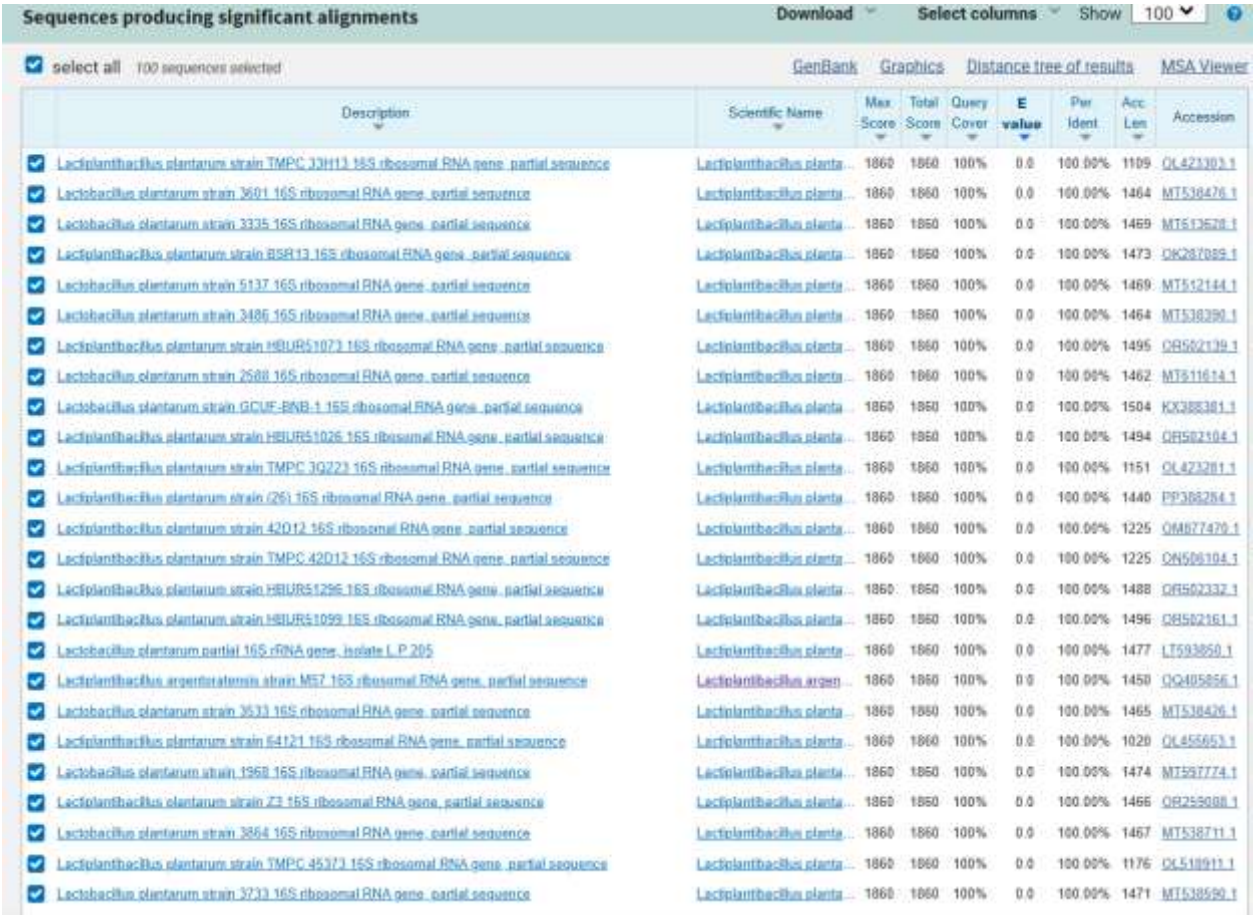
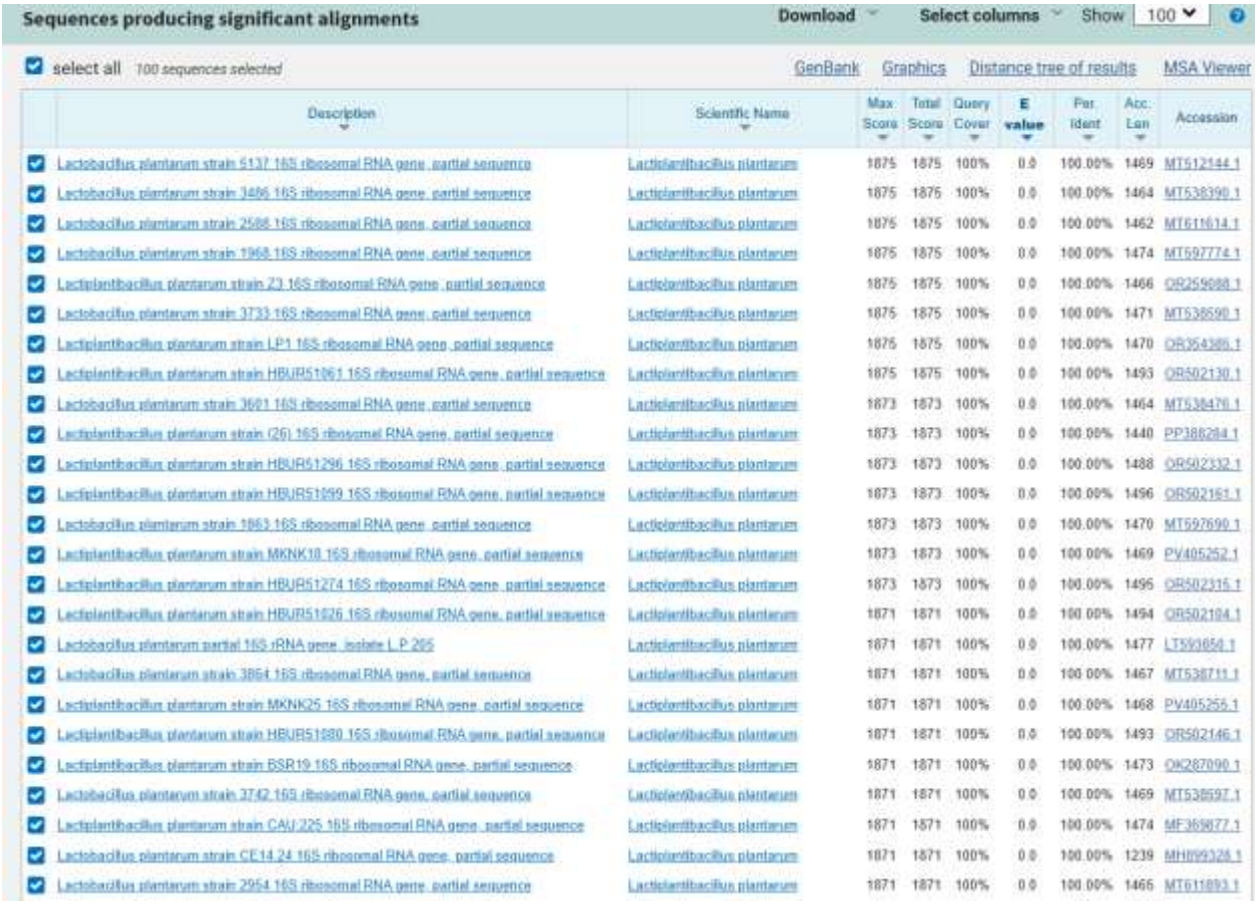


Figure 3.5 – X-1-1 sequence alignment results

Repeat the above operation, input the sequence of X-1-2 to be compared, and BLAST was carried out. The comparison results showed that the strain with the highest similarity to X-1-2 was *Lactobacillus plantarum* (Fig. 3.6), and the Query Cover value was 100 %. X-1-2 was identified as *Lactobacillus plantarum*.



Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lactobacillus plantarum strain 5137 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1875	1875	100%	0.0	100.00%	1469	MT512144.1
Lactobacillus plantarum strain 3486 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1875	1875	100%	0.0	100.00%	1464	MT538390.1
Lactobacillus plantarum strain 2588 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1875	1875	100%	0.0	100.00%	1462	MT611614.1
Lactobacillus plantarum strain 1968 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1875	1875	100%	0.0	100.00%	1474	MT597774.1
Lactiplantibacillus plantarum strain Z3 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1875	1875	100%	0.0	100.00%	1466	OR259088.1
Lactobacillus plantarum strain 3733 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1875	1875	100%	0.0	100.00%	1471	MT538598.1
Lactiplantibacillus plantarum strain LP1 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1875	1875	100%	0.0	100.00%	1470	OR354386.1
Lactiplantibacillus plantarum strain HBUR51061 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1875	1875	100%	0.0	100.00%	1493	OR502130.1
Lactobacillus plantarum strain 3601 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1873	1873	100%	0.0	100.00%	1464	MT538476.1
Lactiplantibacillus plantarum strain (26) 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1873	1873	100%	0.0	100.00%	1440	PP388284.1
Lactiplantibacillus plantarum strain HBUR51296 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1873	1873	100%	0.0	100.00%	1488	OR502232.1
Lactiplantibacillus plantarum strain HBUR51099 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1873	1873	100%	0.0	100.00%	1496	OR502161.1
Lactobacillus plantarum strain 1863 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1873	1873	100%	0.0	100.00%	1470	MT597690.1
Lactiplantibacillus plantarum strain MKNK18 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1873	1873	100%	0.0	100.00%	1469	PV405252.1
Lactiplantibacillus plantarum strain HBUR51274 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1873	1873	100%	0.0	100.00%	1496	OR502315.1
Lactiplantibacillus plantarum strain HBUR51026 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1494	OR502104.1
Lactobacillus plantarum partial 16S rRNA gene, isolate L P 265	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1477	LT591656.1
Lactobacillus plantarum strain 3864 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1467	MT538711.1
Lactiplantibacillus plantarum strain MKNK25 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1468	PV405255.1
Lactiplantibacillus plantarum strain HBUR51680 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1493	OR502146.1
Lactiplantibacillus plantarum strain BSR19 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1473	OK287090.1
Lactobacillus plantarum strain 3742 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1469	MT538597.1
Lactiplantibacillus plantarum strain CAU 225 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1474	MF369877.1
Lactobacillus plantarum strain CE14.24 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1239	MH899326.1
Lactobacillus plantarum strain 2954 16S ribosomal RNA gene, partial sequence	Lactiplantibacillus plantarum	1871	1871	100%	0.0	100.00%	1466	MT611893.1

Figure 3.6 – X-1-2 sequence alignment results

The alignment results showed that the strain with the highest similarity to X-4-1 was Levi *Lactobacillus brevis* (Fig.3.7), and the Query Cover value was 100 %, which determined that X-4-1 was *Lactobacillus brevis*.

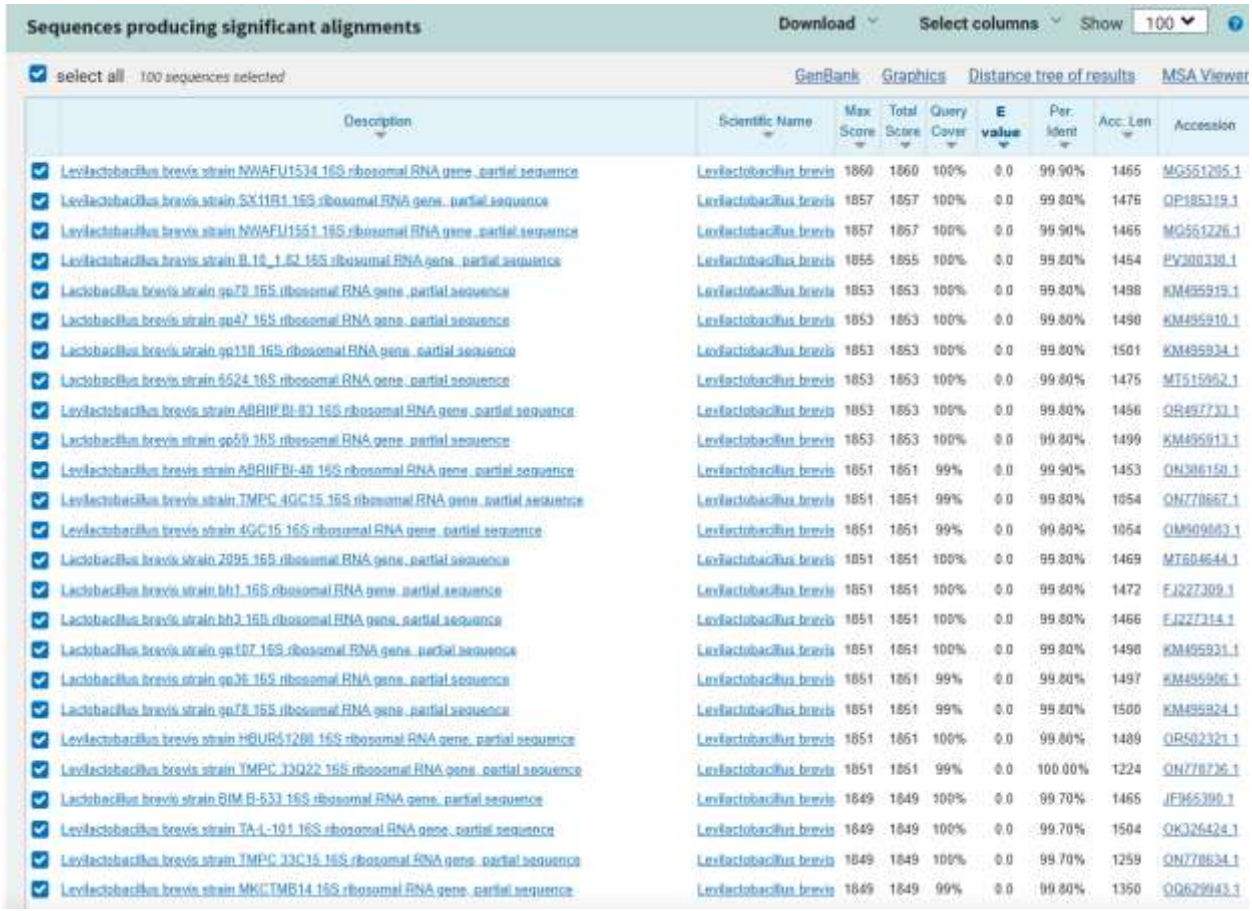


Figure 3.7 – X-4-1 sequence alignment results

The BLAST operation was repeated many times, and the screened strains were identified in turn. The identification results are shown in tab. 3-1.

Table 3.1 – **Strain sequencing identification results**

Strain number species strain number species	Strain number species strain number species	Strain number species strain number species	Strain number species strain number species
X-1-1 *Lactobacillus plantarum* X-1-2 *Lactobacillus plantarum*	X-1-1 *Lactobacillus plantarum* X-1-2 *Lactobacillus plantarum*	X-1-1 *Lactobacillus plantarum* X-1-2 *Lactobacillus plantarum*	X-1-1 *Lactobacillus plantarum* X-1-2 *Lactobacillus plantarum*
X-4-1	X-4-1	X-4-1	X-4-1

Strain number species strain number species	Strain number species strain number species	Strain number species strain number species	Strain number species strain number species
Lactobacillus pumilus X-4-2 *Lactobacillus* pumilus	*Lactobacillus* pumilus X-4-2 *Lactobacillus* pumilus	*Lactobacillus* pumilus X-4-2 *Lactobacillus* pumilus	*Lactobacillus* pumilus X-4-2 *Lactobacillus* pumilus
X-6-1 *Lactobacillus fermentum* X-6-2 *Lactobacillus fermentum*	X-6-1 *Lactobacillus fermentum* X-6-2 *Lactobacillus fermentum*	X-6-1 *Lactobacillus fermentum* X-6-2 *Lactobacillus fermentum*	X-6-1 *Lactobacillus fermentum* X-6-2 *Lactobacillus fermentum*
X-7-1 *Enterococcus faecalis* X-7-2 *Enterococcus faecalis*	X-7-1 *Enterococcus faecalis* X-7-2 *Enterococcus faecalis*	X-7-1 *Enterococcus faecalis* X-7-2 *Enterococcus faecalis*	X-7-1 *Enterococcus faecalis* X-7-2 *Enterococcus faecalis*
X-10-1 *Lactobacillus paracasei* X-10-2 *Lactobacillus paracasei*	X-10-1 *Lactobacillus paracasei* X-10-2 *Lactobacillus paracasei*	X-10-1 *Lactobacillus paracasei* X-10-2 *Lactobacillus paracasei*	X-10-1 *Lactobacillus paracasei* X-10-2 *Lactobacillus paracasei*

The sequencing sequences of strain X-1-1 and strain X-2-1 belonging to *Lactobacillus plantarum* were sorted into a file and stored in fasta format for sequence alignment (Fig.3.8).

Figure 3.8 – X-1-1, X-1-2 sequence alignment

The sequencing sequences of X-1-1, X-1-2, X-4-1, X-4-2, X-6-1, X-6-2, X-7-1, X-7-2, X-10-1 and X-10-2 were sorted into the same file and saved in fasta format. The phylogenetic tree was constructed using MEGA 11 software (Fig. 3.9). Through the phylogenetic tree, the genetic relationship between different strains can be seen.

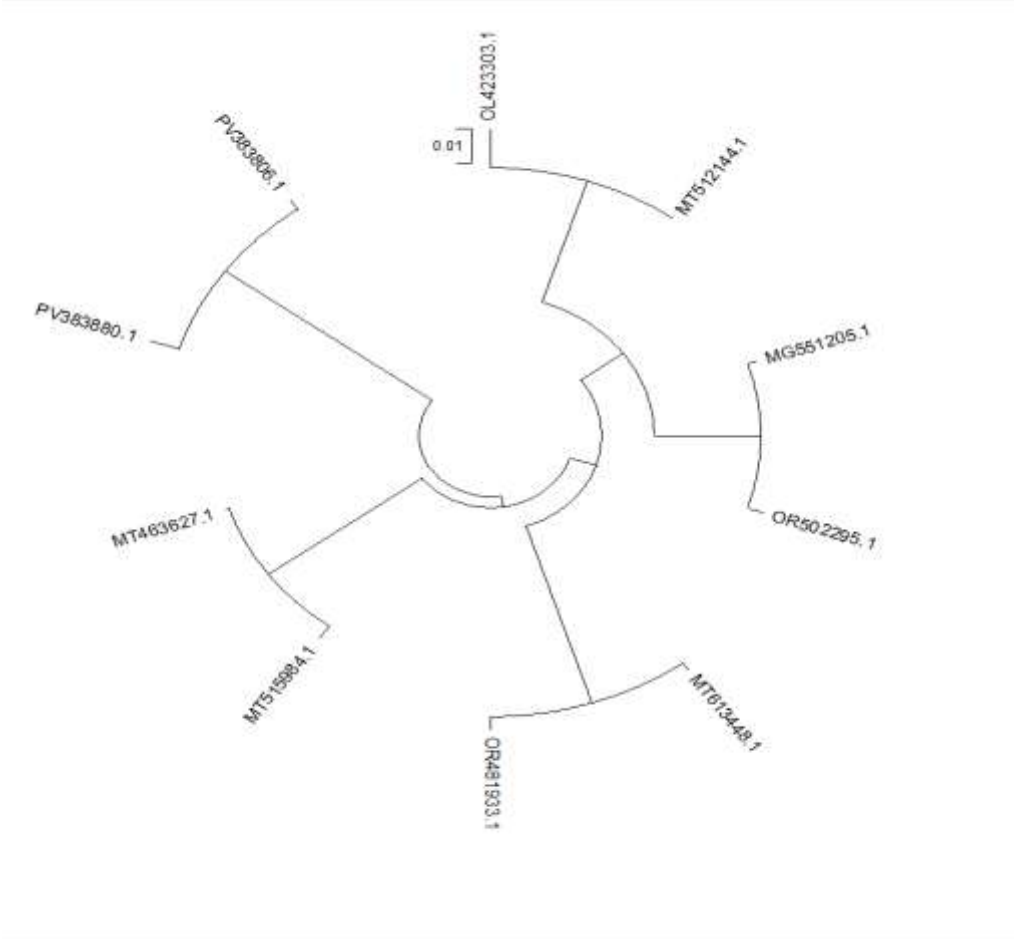


Figure 3.9 – Phylogenetic tree

Conclusions to chapter 3

1. The PCR amplification products were sequenced to obtain the sequencing map.

2. The sequence to be aligned was compared with the sequence in the database to obtain the sequencing results for product analysis.

3. The phylogenetic tree was constructed, and the genetic relationship between different strains was seen through the phylogenetic tree.

CONCLUSIONS

1. As a kind of microorganisms widely distributed in nature, lactic acid bacteria have high application value in important fields closely related to human beings. As a probiotic, it has many physiological functions such as regulating intestinal flora balance, immune regulation, metabolic regulation, and anti-tumor. In this study, the isolation, screening and molecular identification of lactic acid bacteria in environmental samples were systematically explored. Five different lactic acid bacteria were isolated from environmental samples, and 10 strains of lactic acid bacteria were selected by MRS medium and other physiological and biochemical experiments. The classification information of these 10 strains of lactic acid bacteria was obtained by 16 S r DNA sequence alignment. The obtained sequencing sequence was compared with the sequence in the NCBI database. The sequence analysis showed that the lactic acid bacteria isolated from the environmental samples were *Lactobacillus plantarum, Lactobacillus* pumilus, *Lactobacillus fermentum*, *Enterococcus faecalis* and *Lactobacillus paracasei*. The phylogenetic tree was constructed by MEGA to understand the evolutionary relationship between strains and determine the genetic relationship of 10 strains of lactic acid bacteria.

2. Lactic acid bacteria can directly regulate the balance of intestinal flora and microbial environment. Its metabolites can improve specific immunity, prevent and alleviate diarrhea, regulate intestinal microflora, regulate immunity, and alleviate allergies. In addition, lactic acid bacteria also help to treat a variety of diseases, such as gastrointestinal diseases, immune diseases and inflammatory bowel disease. When lactic acid bacteria are added to foods, they enhance the host 's immune system response, help digestion, and regulate the gastrointestinal microbiota. Lactic acid bacteria such as lactobacilli and enterococci are known as probiotics because they are thought to reduce diseases such as diarrhea, inflammatory bowel disease and irritable bowel syndrome.

3. Due to the widespread use of lactic acid bacteria as starter cultures in food production, the preservation and improvement of the flavor of various dairy products, meat, vegetables and grain products, lactic acid bacteria have a great market impact. As a functional food ingredient with potential health benefits, the market for lactic acid bacteria has also increased due to people 's increasing interest in probiotics. Lactic acid bacteria have many uses, including food and dairy industries, as probiotics to enhance human and animal health ; as a biological fertilizer, and as a source of extracellular polysaccharides in various industries.

4. Lactic acid bacteria are recognized as one of the most commonly used microbial types in the world for the development of dairy and non-dairy fermented foods due to their low price, easy cultivation, storage and use, and simple collection. Even now, this group of microorganisms is still one of the most studied microorganisms. Because lactic acid bacteria have the ability to inhibit bacterial growth and promote plant growth, they can replace inorganic fertilizers and pesticides.

5. Starch films were added by lactic acid bacteria to protect fruits and vegetables from oxidative damage. By using this strategy, the shelf life can be extended without compromising the established standards of the food packaging process. Lactic acid bacteria strains inhibit pathogenic microorganisms and improve animal health through nutrition, which makes it extremely beneficial to animal health.

6. A large number of studies have shown that lactic acid bacteria can be isolated from feed, inhibit pathogenic pathogens, and support the gut microbiota of humans and animals. In addition, lactic acid bacteria are increasingly used commercially in the synthesis of chemicals, drugs and other beneficial commodities. Recent studies have shown that the synthesis of lactic acid by biotechnology can reduce the environmental pollution of the petrochemical industry. These new technologies will improve productivity and promote the sustainable development of agriculture and animal husbandry in the future.

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