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SCREENING AND IDENTIFICATION OF AN ANTIBACTERIAL AGENT-PRODUCING *LACTOBACILLUS* STRAIN AND ITS APPLICATION IN BANANAS PRESERVATION

– Research paper –

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Abstract: With the development and progress of human society and the continuous enhancement of people's health awareness, lactic acid bacteria inhibitors as a natural biopreservative have an irreplaceable position, with the advantages of high efficiency, broad spectrum, stability, safety and other characteristics. This paper studied the antibacterial effect of 23 lactic acid bacteria; the strains with the best antibacterial effect was identified by 16S rDNA method as being *Lactobacillus rhamnosus* L57 and was applied to banana storage. Our study showed that *Lactobacillus rhamnosus* L57 has a broad spectrum, inhibiting *Escherichia coli*, *Staphylococcus aureus*, *Vibrio alginolyticus* and *Bacillus nato*. Excluding the interference of organic acids, hydrogen peroxide and extracellular polysaccharide, the salting out of ammonium sulfate preliminarily determined that the proteins or peptides are the antibacterial substances in the antibacterial agent. Physicochemical properties showed that the microorganism had good antibacterial activity after trypsin, pepsin and papain, with good tolerance to pH, temperature and UV light, and the pH optimal range of 2.0-4.0. The mixture of freeze-dried powder of *L. rhamnosus* L57, chitosan and natamycin was used for banana preservation, with the proportion of freeze-dried powder of 1.25%, chitosan 1.5% and natamycin 0.03%. The preservation period was extended by 4 days compared with the absence of either bacteriostatic agent.

Keywords: *Lactobacillus rhamnosus*, bananas, probiotic antibacterial agent, biopreservative

INTRODUCTION

Food safety has attracted more and more attention. As one of the critical aspects of food safety, ensuring the nutrition and health of food by extending its shelf life and improving hygiene quality through food preservation technology is particularly important (García et al., 2010). With the globalization of the food market and the introduction of new foods, there is an increasing demand for new manufacturing techniques. For example, some fresh sliced or ready-to-eat foods may need higher standard preparation techniques to prevent food safety risks caused by microbial contamination (Du Gengan, et al., 2022).

According to CDC estimates, there are approximately 47.8 million cases of foodborne illness, 127,839 hospitalizations and 3,037 deaths (including those caused by known and unknown pathogens) in the United States each year (Scallan, et al., 2011). Foodborne pathogenic bacteria, characterized by diverse sources and rapid propagation in food, become the primary food safety hazard factor (Velusamy et al., 2009). Foodborne diseases are one of the serious diseases endangering public health in the world. Common foodborne diseases are caused by *campylobacter*, *Salmonella*, *listeria* and *viruses* (Zhang, et al., 2013). Therefore, this paper mainly studies how to inhibit the growth of pathogenic microorganisms in food.

Pathogenic microorganisms are the main cause of food spoilage (O'Brien, 2012). At present, chemical, physical and microbial storage methods are usually used to extend the shelf life of food at home and abroad.

Physical storage extends the shelf life of food by changing environmental conditions, gas composition and other physical methods (Woods et al., 1999), which can be mainly divided into quick-freeze storage, air-conditioned storage, cold-

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sterilization storage, etc. (Kubo et al., 1990). Physical storage has the advantages of safety, cleanliness and large quantity without using any preservatives, but it has great damage to the internal structure of food, low energy utilization rate and high cost.

Chemical preservation refers to the use of chemical antibacterial agents to prevent food deterioration, mainly including benzoic acid and its sodium salt, sorbic acid and its sodium salt, propionic acid and its salts, dehydroacetic acid, sulfite, nitrite, etc. (Lone Grama, et al., 2002). Chemical bacteriostatic agent is the most commonly used in life, with the advantages of high efficiency, low dosage, low price and easy production. However, they are known to be toxic and can pose carcinogenic and mutagenic hazards as they can remain in the body.

Biopreservation refers to use of biological inhibition to extend the shelf life of food. Biological bacteriostatic agents refer to natural bacteriostatic substances extracted from animals, plants and microorganisms or modified by bioengineering technology for human safety. In daily life, biological antibacterial agents from animals and plants mainly include protamine, antibacterial peptides, lysozyme, chitosan, propolis, Chinese herbal substances, spices, plant polysaccharides, plant proteins, polyphenols, etc. (Poveda et al., 2018; Hugo et al., 2015). Bacteriostatic production of microorganisms is a current research hotspot, and probiotics have attracted much attention. The metabolites produced by probiotics in the growth and reproduction process, such as bacteriocin, lysozyme, protease, hydrogen peroxide, organic acids and other substances (Leroy F, et al., 2004), have a certain inhibitory effect on the growth of spoilage bacteria and pathogens. When applied to food, shelf life can be extended (Gulahmadov et al., 2009; Leroy et al., 2005). Probiotics are microorganisms colonizing the human intestinal tract and are beneficial to the host (WHO, 2001). The lactic acid bacteria (LAB), generally considered as 'food-grade', show special promise for selection and implementation as protective cultures (Holzapfel,et al., 1995). Because LAB antibacterial agent can inhibit microbial growth and has the advantages of high efficiency, broad spectrum and convenient use. Therefore, in this paper, probiotics were selected to product biological preservation. The probiotics producing bacteriostatic agents were firstly screened and re-screened. At the same time, strain identification was carried out. Finally, the identified microorganism was applied to banana preservation.

MATERIALS AND METHODS

Strains and materials

Test strain: *Penicillium chrysogenum*, *Aspergillus niger, Saccharomyces boulardii*, *Saccharomyces cerevisiae, Vibrio alginolyticus*、*Escherichia coli*、*Staphylococcus aureus, Bacillus natto* and 23 strains of *Lactobacillus* were provided by Department of Bioengineering, College of Food Science and Engineering, Shaanxi University of Science and Technology. Bananas are bought in supermarkets.

Culture media

MRS medium was used for activation and counting of probiotics. LB medium for activation culture and antibacterial test of *Escherichia coli*, *Vibrio alginolyticus, Bacillus natto* and *Staphylococcus aureus*; Modified Martin medium was employed for liquid activation culture of fungi *Penicillium chrysogenum, Aspergillus niger, Saccharomyces boulardii* and *Saccharomyces cerevisiae*.

Main reagents and instruments

The bacterial genomic DNA rapid extraction kit, proteinase K, DNA Marker, universal primers 27F and 1492R, were used, as provided by Suzhou Jinweizhi Biotechnology Co., Ltd. Other deiveces were: PCR amplifier; pH meter; electric thermostatic incubator; microscope; portable steam sterilizer; spectrometer; high speed desktop centrifuge; vacuum freeze dryer; electronic balance; vertical shock incubator; ultra-clean work table.

Preparation of the indicator bacterial suspension

The bacterial solutions of activated *E. coli*, *S. aureus*, *V. alginolyticus*, and *B. natto* were inoculated into LB liquid medium and incubated at 37℃ for 20h. The bacterial content of the bacterial suspension was calculated by the dilution coating plate method, and 10^6 CFU / mL was used as the indicator bacterial suspension concentration.

Strain activation and culture

23 probiotic powder stored at low temperature (-18℃) were added into MRS broth medium for activation, 24 h in an incubator at 37℃, and then 5% inoculum amount was cultivated into MRS broth medium for two generations. Finally, the activated probiotics were refrigerated in a refrigerator of 4℃. *E. coli*, *S. aureus*, *B. natto* and *V. alginolyticus* were cultured in LB liquid medium at 37℃ for 24 h. After 2 generations of activation, different gradients were diluted in 0.9% saline and stored in refrigerator of 4℃. *P. chrysogenum*, *A. niger, Sacch. boulardii*, and *Sacch. Cerevisiae* were cultured at 30℃ for 24 h using modified Martin medium, after activation for 2 generations, and different gradients were diluted

in 0.9% saline and stored in 4℃ freezer for reserve.

Preparation of bacteria and antibacterial agents
Probiotics were inoculated in MRS broth medium at 5% inoculum, then cultured at 37℃ for 24 h, centrifuged for 15 min at 8000 r/min. The bacteria and bacteriostasis (fermentation supernatant) were collected. The supernatant was filtered by 0.45 μ m microporous filter membrane and kept in 4℃ refrigerator.

Screening of the antimicrobial-producing probiotics
Primary screening was carried out, using *E. coli, S. aureus, V. alginolyticus and Penicillium* as the indicator bacteria in the laboratory to indicate the antibacterial activity of the bacteria, and the diameter of the pore was 9 mm. The probiotics of the screened inhibitor were rescreened by the pH and acidity of the inhibitor, the wet weight of the bacteria and the diameter of the antibacterial action.

Morphological identification of Lactobacillus sp
In the superclean workbench, the selected strains after activation were picked with the inoculation needle, inoculated on the solid MRS medium plate, and cultured anaerobically in a 37℃ incubator for 48h to observe the colony characteristics. Meanwhile, the activated strains were vacuum freeze-dried with 20% skim milk and further observed the morphological characteristics of the bacteria under scanning electron microscope.

Identification of bacteriostatic strains by 16S rDNA
The 16S rRNA gene of unknown strain was amplified by PCR using 16S rDNA primers, DNA sequencing, homology comparison with known sequences in GenBank, determination of bacterial species, and classification of bacteria into genus or species (Abubakr, et, al, 2017).

Determination of bacteriostatic and fungistatic agents
For the determination of antimicrobial spectrum, indicator microorganisms were selected, including gram-negative bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus natto*, and *V. alginolyticus*, the fungi *Penicillium chrysogenum*, *Aspergillus niger, Saccharomyces boulardii* and *Saccharomyces cerevisiae*. The antibacterial activity of the inhibitor against the indicator strains was determined by punching method; the antimicrobial coil diameter was measured by the vernier caliper and the results were recorded.

Strain growth curve and antibacterial activity curve determination
The activated probiotics were inoculated in MRS broth, incubated at 37℃, and the OD 600 value and pH value were measured every 4h. The culture solution was collected by a centrifuge at 8000 r/min. *E. coli* and *S. aureus* were used as the indicator bacteria to detect the antibacterial activity of the antibacterial agent.

Deinhibition of each component of fermentation broth
Probiotics can produce a variety of biological active substances, including bacteriocins, organic acids, hydrogen peroxide, extracpolysaccharide, etc., therefore, in order to initially determine the bacteria bacteriostatic material composition, using agar diffusion method to detect hydrogen peroxide, organic acids, polyspolysaccharide after antibacterial activity, reuse ammonium sulfate with antibacterial substances in fermentation broth for crude extraction.

Determination of the antimicrobial agent stability
The study of the stability of probiotic antibacterial agents can preliminarily determine the subsequent preparation, storage and application conditions, so the thermal stability, UV stability, pH stability and protease sensitivity of probiotic antibacterial agents can be studied.

OD_{600nm} values of bacterial medium were determined
After fermentation of probiotics in appropriate medium for a certain time, uninoculated medium was used as the control group, the absorbance was measured by UV spectrophotometer at a wavelength of 600 nm, and OD_{600nm} value and pH value were used as the ordinate to plot the bacterial growth curve as the abscissa.

Thalli wet weight determination
All 18 mL probiotic culture medium were transferred to a sterile centrifuge tube and centrifuged at 8000 r/min for 15 min. The supernatant and the wet bacteria sludge was removed, and then the weight of the bacteria sludge was measured using an electronic balance.

pH value determination
At 25℃, the pH value of the culture medium was determined by using a pH-meter.

Determination of antimicrobial activity
Bacteriostatic experiment with Agar diffusion method (punching method) of probiotic bacteria and bacteriostatic agent (Michaylova Michaela, et, al, 2021), testing its bacteriostatic activity, expressed as a bacteriostatic circle diameter of the activity of antimicrobial agent size, hole diameter of 9 mm, use vernier caliper to measure the circle of inhibiting, bacteriostatic ring test records include the diameter of the hole diameter size.

Preparation of bacteriostatic freeze-dried powder

The prepared probiotic bacteriostatic agent and appropriate dispersant were freeze-dried for 24 h in a vacuum freeze-drying machine to get bacteriostatic freeze-dried powder.

Preparation of compound preservative

The prepared probiotic antibacterial agent (PAA) was used alone or the combined with the biological antibacterial agent to prepare the preservatives of different formulas, with bananas as the action object, to study its preservation effect. According to correlation studies (Nisia et al,2012; Deegan et al, 2006; Hossain, 2013), 1.5% chitosan (C) and 0.03% natamycin (N) were used; chitosan was dissolved in 3% citric acid, the minimum bactericidal concentration of 0.625% and 1.25% lyophilized powder were used for the preservation of bananas, split into seven groups. The solution 3% citric acid was the group 1 as control (see Table 1).

Determination of microbial quantity in banana preservation

Sensory evaluation: 10 evaluators were selected to score the Browning degree, wilting degree and severity of disease of bananas respectively. The average score of 10 evaluators was taken as follows :9 points: fresh, crisp and tender without Browning and rotting, with good quality; 7 points: crisp and tender, no Browning and rot, good quality; 5 points: slightly wilted, edible; 3 points: wilting, local rot and Browning, not edible; 1: Severe wilting, rot, Browning, not edible.

Shelf life: The shelf life of fruit is influenced by different post-harvest treatments, calculated by calculating the time it takes for the fruit to fully ripen to maintain optimal marketing and dietary quality.

Titration acidity: a known amount of sample was mixed with distilled water and filtered with a volume of 100 mL in a cylinder. A 10 mL filtrate was titrated with 0.1 mol/L sodium hydroxide and phenolphthalein was used as an indicator. The end point of titration was the appearance of pink color and the color did not fade for 30 s. Acidity was calculated using the equation (1).

$$Acidity\ (\%)=(E\times T\times V1\times N)/(V2\times W\times 1000)\times 100 \quad (1)$$

In formula (1): E-The conversion coefficient of acid, malic acid: 0.067; N-sodium hydroxide concentration (mol/L); W-Quantity of the sample (g); V1-Total volume (mL) of the sample; V2-Volume (mL) of the sample at the titration; T-The titration volume is the volume of sodium hydroxide consumed (mL).

Total Soluble solids: Soluble solids include carbohydrates, organic acids and amino acids. The content of soluble solids in bananas was determined using a hand-held sugar meter (Brix).

Microbial count: Bananas with almost the same appearance (weight, shape and color) were selected, dipped in 2.5% hydrogen peroxide for 5 min for disinfection, washed and dried, soaked in the prepared bacteriostatic agent for 1 min, and incubated in 37℃ aerobic environment for 48h. Among them, the number of bacteria was determined by GB 4789.2-2016 and the number of mold by GB 4789.2-2016.

Determination of color: The color change of bananas during storage is determined by spectrophotometer, by measuring L* (represents the brightness of the object, and 0-100 represents the change from black to white); A* (represents the red and green color of the object, positive values represent red and negative values represent green); B* (represents the yellow and blue color of the object, with positive values representing yellow and negative values representing blue).

Total weight loss: Water loss is gas-phase diffusion due to the different pressure of water vapor inside and outside fruits. Weight loss is determined by weighing the mass of samples stored at different times by electronic balance, and calculating the percentage of water loss according to the original mass (day 0), as shown in Equation (2).

$$Weight\ loss\ \% = (initial\ weight - final\ weight)/\ initial\ weight\times 100\% \quad (2)$$

Table 1. The treatment groups of experiment and preservative composition

	Chitosan C (%)	Freeze-dried powder of bacteriostatic agent PAA (%)	Natamycin N (%)	combination
1	0	0	0	Control
2	0	0.625	0	PAA
3	0	1.25	0	2PAA
4	1.5	0	0	C
5	0	1.25	0.03	2PAA+N
6	1.5	0	0.03	C+N
7	1.5	1.25	0.03	C+2PAA+N

RESULTS AND DISDUSSION

The antibacterial properties of probiotic bacteria and supernatants

The activated probiotics were added to MRS broth at 5% (v/v) and cultured at 37℃ for 24 h, centrifuged at 8000 r/min for 15 min to obtain supernatant and sludge. The sludge was suspended in normal saline, and *S. aureus* and *E. coli* were used as indicator bacteria. The results of the antibacterial activity test are shown in Figure 1. The supernatant showed a distinct inhibitory activity against the indicator bacteria. The antibacterial performance of probiotic strains L20, L21 and L33 are weaker than L57. LX, L20 and L21 against *E. coli* has bigger inhibitory circles than *S. aureus*. The inhibion areas for L20, L21, and L33 against *E. coli* and *S. aureus* are 14.32-15.84 mm and 12.13-16.17mm, respectively. L57 and LX exhibit 16.57-16.91 mm and 18.85-19.44 mm inhibition against *E. coli* and *S. aureus*, respectively. The antibacterial performance of probiotic antibacterial mainly comes from the antibacterial activity of the fermentation broth, which was studied in subsequent experiments.

Primary screening of Antibacterial Agent-producing *Lactobacillus*

The results of 23 *L. probiotic* strains cultured in MRS broth based on 37℃ for 24 h, using *E. coli, S. aureus, V. alginolyticus* and *P. chrysogenum* as the indicator microorganims, and their antimicrobial activity are shown in Figures 2, 3 and 4.

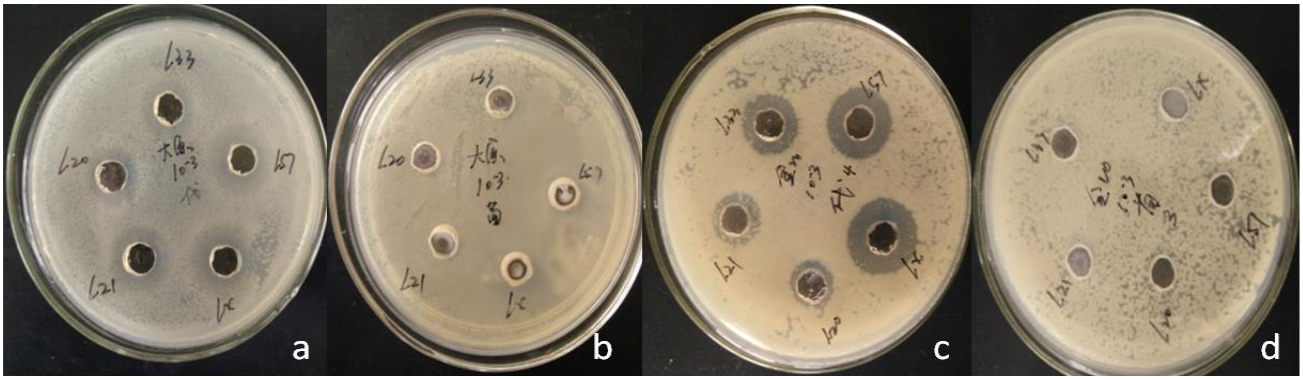


Figure 1. Bacteriostatic result of probiotic bacteria and supernatant (Antibacterial activity of fermentation supernatant against *E. coli (a)* and *S. aureus* (c); antibacterial activity of bacteria against *E. coli* (b) and *S. aureus* (d))

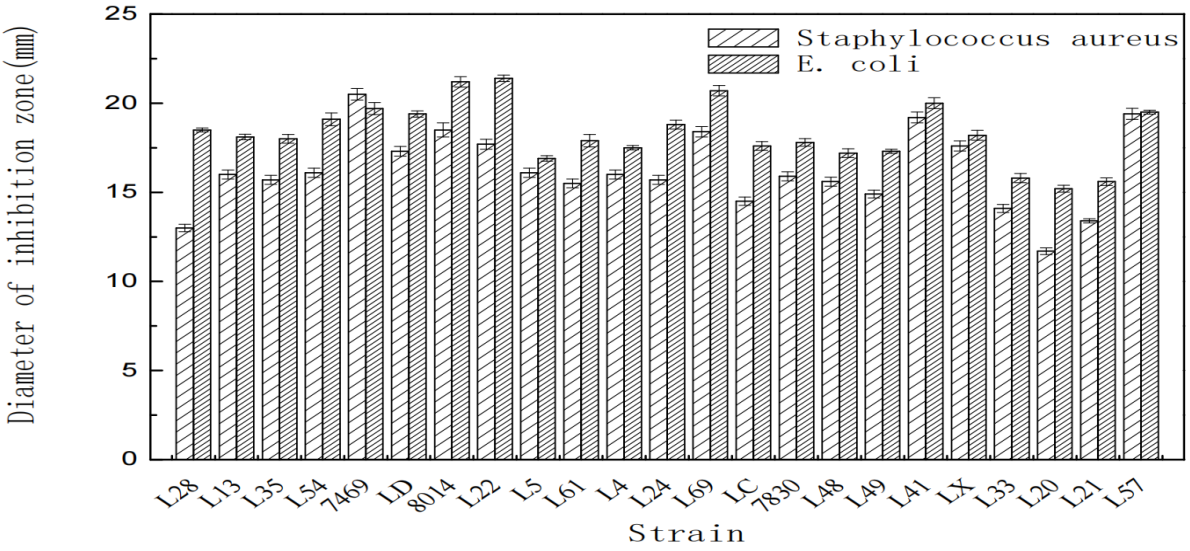


Figure 2. Antibacterial effect of *Lactobacillus* on *S. aureus* and *E. coli*.

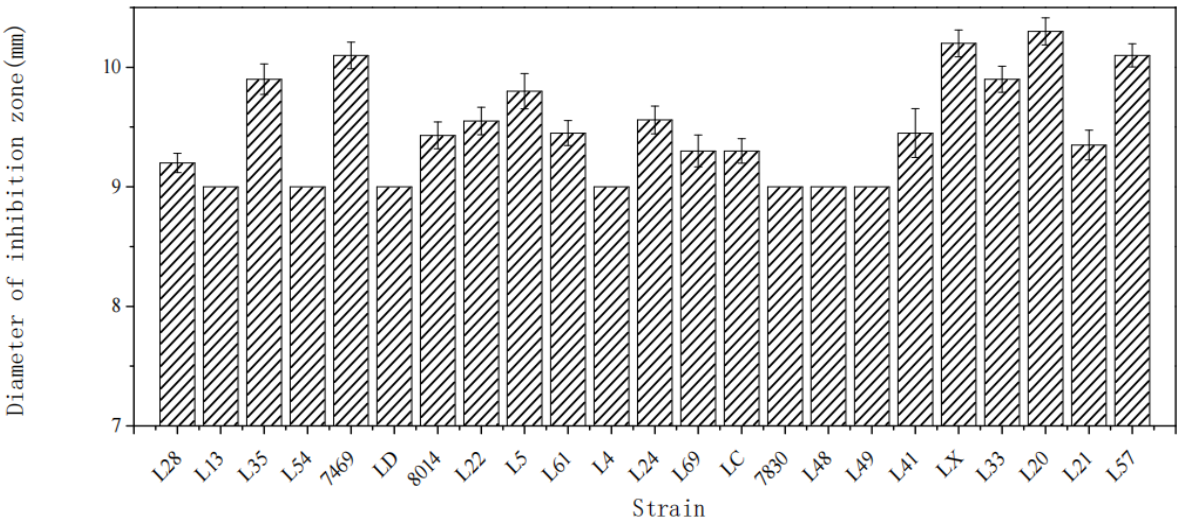


Figure 3. Antifungal effect of probiotic agents on *Penicillium chrysogenum* strain

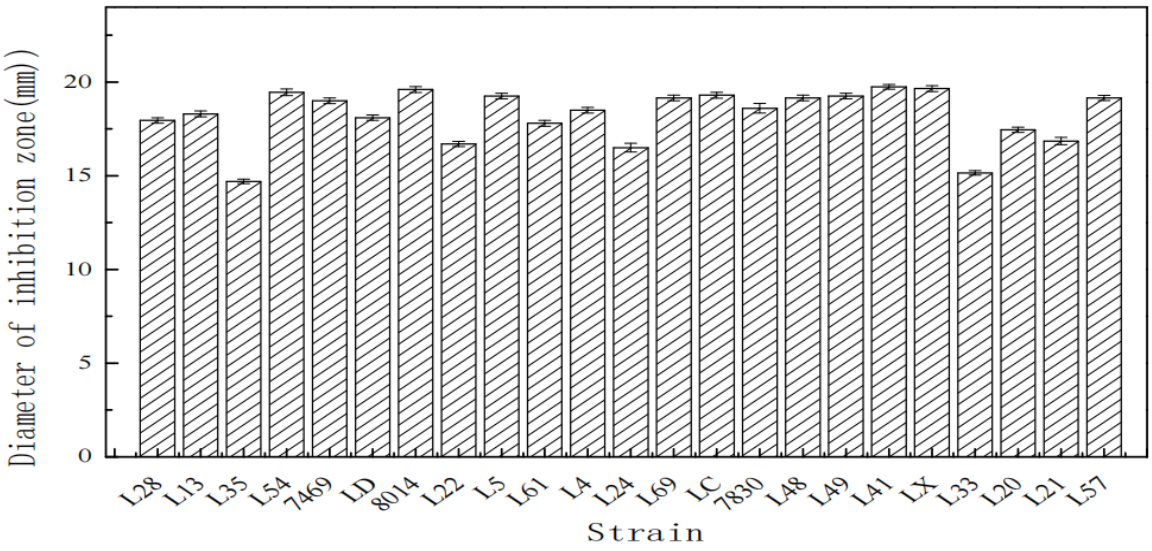


Figure 4. Antibacterial effect of probiotic antibacterial agents on *Vibrio alginolyticus* strain

Figure 1 shows that the bacteriostatic agent acts against both *E. coli* and *S. aureus*; for the *E. coli* strains, the diameter of the inhibitory coil is ranked as L69 < 8014 < L41 < L57 <7469. Against *S. aureus*, the coil diameter was ranked as L57 < 7469 < L41 < 8014 < L22 < L69.

Penicillium is a class of omnivorous fungi which can grow on any matrix containing organic matter; *Penicillium* is usually on citrus and other fruits, refrigerated cheese and some other foods can be widely alive, heat resistance is not easy to control. As known from Figure 2, the antimicrobial agents produced by some probiotics were not effective on *Penicillium*, and strains with bacteriosphere diameter greater than 9.5 mm were ranked L22, however, the antifungal coil diameter of L69, L41, and 8014 ranged from 9.3 to 9.5 mm, it is possible that some antimicrobial agents of probiotics contain substances inhibiting *Penicillium*.

Figure 3 shows that probiotic inhibitors can inhibit the growth of *V. alginolyticus*; strains with bacteriosphere diameter greater than 19.0 mm were sorted as 7469 < L57 < L48 < L69 < L5 < L49 < LC < L41 < 8014 < L54 < LX.

By analyzing the results, the strains with significant inhibition of the indicator bacteria *E. coli*, *S. aureus* (as the main indicator bacteria) and *P. chrysogenum* and *V. alginolyticus (*as additional microoganisms) were 7469, L57, L22, L41. These four strains were selected for further studies.

Rescreening of Antibacterial Agent-producing *Lactobacillus*

For further screening of strains with obvious inhibitory effect on the indicator bacteria, a 5% inoculum in MRS broth was used, cultured at 37℃ for 24 h; every 6h sampling its wet weight, pH, acidity, and antibacterial coil diameter were

measured, as shown in Figure 5. pH, acidity and wet weight of probiotics number L41 changed the slowest with time, indicating that the slowest growth of the bacteria. Little change with time, and the index value of bacteria L57 is faster with the extension of fermentation time compared with L22 and 7469, indicating that L57 is more suitable for bacteria as an antibacterial agent. After 24 h of fermentation, the antibacterial agent produced by L57 has the strongest antibacterial ability and the largest acidity, at this time, pH, wet weight, acidity and antibacterial coil diameter were 3.76,221 T, 0.7521 g and 15.25 mm, respectively. After 24 h of fermentation, L22 pH was 3,3.72 and 7469 wet weight was 0.9194 g. Comprehensive evaluation, strain L57 was better than strains L22 and 7469, so L57 as the best strain in subsequent experiments.

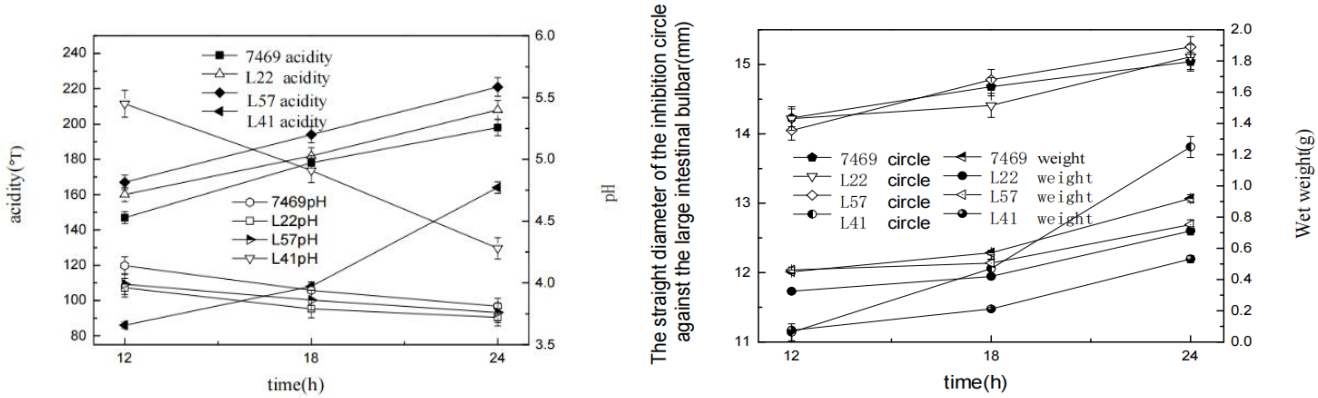


Figure 5. Effect of culture time on growth of probiotics, antibacterial effect, pH and acidity

Body and colony morphology of strain L57

From the strain L57 in the logarithmic growth period, an appropriate amount of bacterial solution was collected with an inoculated needle and streaked in MRS agar in 37℃ for 24 h to observe the colony morphology, microscopy was taken and the lyophilized bacterial powder was observed under scanning electron microscope. The colony morphology and body morphology of L57 are shown in Figure 6. The L57 is rod-shaped, with different colony sizes, shiny, round, milky white, neat edges, smooth and moist surface, viscous and easy to pick, which is in line with the colony morphology characteristics of bacteria.

Identification result of *Lactobacillus* using 16S rDNA

The 16S rDNA genes from the unknown strains were PCR amplified with primers at both ends of the 16srRNA and subjected to DNA sequencing and compared to known sequences in GenBank. The filtered L57 was identified with 16SrDNA, and the target fragments were amplified by PCR and subjected to agarose gel electrophoresis, as shown in Figure 7. The 16R sDNA gene sequence of *Lactobacillus* L57 in Figure 8.

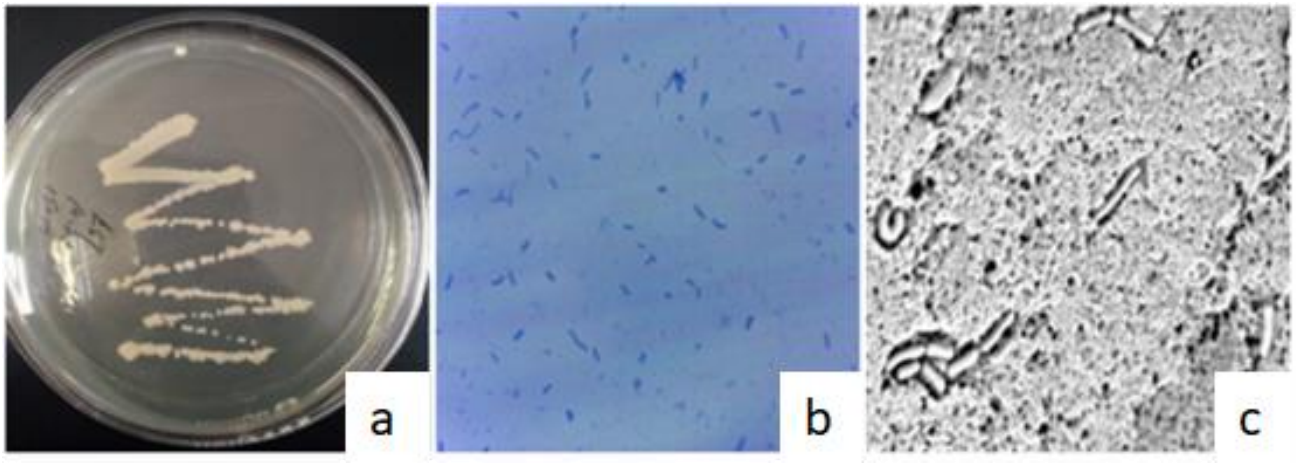


Figure 6. L57 colony and cell morphology (a, b and c are colony morphology, optical microscope and scanning electron microscopy bacterial morphology, respectively)

The rDNA sequencing of 16S, DNA form results of DNA base sequence and DNA sequences were aligned in the NCBI database to establish the phylogenetic tree, as shown in Figure 9. Strain L57 shares 100% homology with *L. rhamnosus* NBRC 3425 and *L. rhamnosus* JCM 1136, indicating that strain L57 is *L. rhamnosus*.

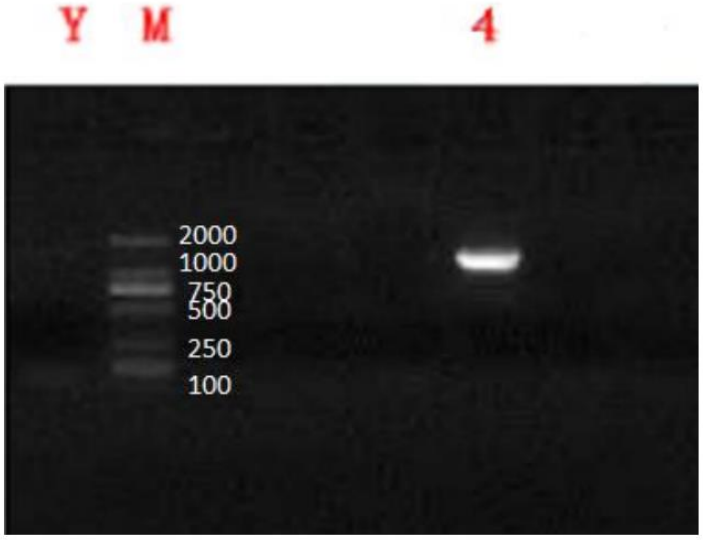


Figure 7. Results of agarose gel electrophoresis (4 is L57; M is Marker (DL2000：2000、1000、750、500、250、100bp);Y is Negative control)

TGCAGTCGAACGAGTTCTGATTATTGAAAGGTGCTTGCATCTTGATTTAATTTTGAACGAGTGGC
GGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATG
CTAATACCGCATAAATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTT
TGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAATGATACGTA
GCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAA
GAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTC
GGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTG
ATGTGAAAGCCCTCGGCTTAACCGAGGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAA
GAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGC
GAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATT
AGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCA
GTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAA
GGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGA
ACCTTACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAAT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA
ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTG
CTACAATGGATGGTACAACGAGTTGCGAGACCGCGAGGTCAAGCTAATCTCTTAAAGCCATTCT
CAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAG
CACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTA
ACACCCGAAGCCGGTGGTCGTAACCC

Figure 8. 16R sDNA gene sequence of *Lactobacillus* L57

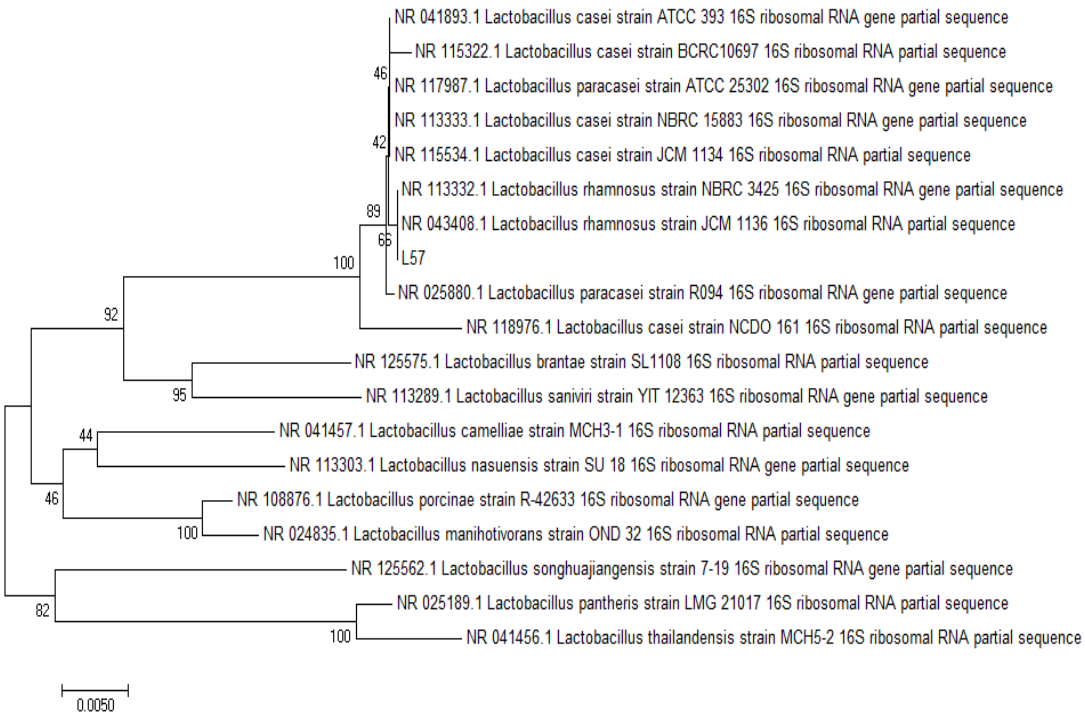


Figure 9. Phylogenetic tree of *Lactobacillus* L57

Determination of the antimicrobial profile of bacteriostasis agents

Eight indicator strains were selected, four bacteria strains and four fungi. *Lactobacillus rhamnosus* L57 was detected for the production of bacteriostatic activity against the eight strains of indicator bacteria; the antibacterial test results as shown in Table 2, found that for *S. aureus*, *Bacillus natto*, *E. coli* have strong inhibitory effect and weak inhibitory activity, to *Penicillium chrysogenum*, *Aspergillus niger, Saccharomyces boulardii* and *Saccharomyces cerevisiae.*

Content in hydrogen peroxide and organic acids of *L. rhamnosus* strain

To determine whether *L. rhamnosus* producers contains hydrogen peroxide, 10 mg/mL peroxygenase 1 mL mixed with 1 mL bacteriostatic agent, Untreated bacteriostatic agent served as a control. The antibacterial activity was determined by punching method using *Staphylococcus aureus* and *E. coli* as indicator bacteria, The results are shown in Figure 10. The diameter of the *E. coli* treated and untreated inhibitors were 18.29 ± 0.12 mm and 18.34 ± 0.24 mm, respectively. The diameters against *S. aureus* were 18.67 ± 0.23 mm and 18.71 ± 0.15 mm, respectively. The antibacterial activity of treated to untreated bacteriostatic agents show that the prepared bacteriostatic agent does not contain hydrogen peroxide.

To determine whether the bacteriostatic agents produced by *L. rhamnosus* contain organic acids. *S. aureus* and *E. coli* were used as indicators. Results are shown in Figure 11. No inhibitory circle in regions a and b are identified in Figure 11. However, the c region has a significant antibacterial circle. The diameter sizes of the inhibitory coil for *E. coli* and *S. aureus* were 18.51 ± 0.17 mm and 18.30 ± 0.22 mm, respectively, suggesting that the antibacterial agent does not contain the fermentation product organic acids.

Table 2. Determination of the antimicrobial spectrum of antibacterial agents produced by *L.rhamnosus*

Indicator	Antibacterial activity*
Escherichia coli	+++
Staphylococcus aureus	++++
Vibrio alginolyticus	++++
Bacillus natto	+++
Penicillium chrysogenum	+
Aspergillus niger	-
Saccharomyces boulardii	-
Saccharomyces cerevisiae	-

*: ++++ indicates the diameter of the coil of 20-25mm; +++ indicates the diameter of 15-20mm; ++ indicates the diameter of 10-15mm; + indicates the diameter of <10mm; -indicates the occurrence of no coil

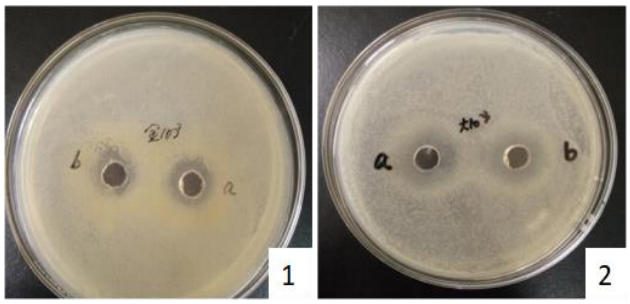


Figure 10. Antibacterial test results of antibacterial agents with catalase(1 and 2 represent *S. aureus* and *E. coli* for the indicator bacteria antibacterial test, respectively; a and b represent untreated and antibacterial agents was treated by catalase, respectively)

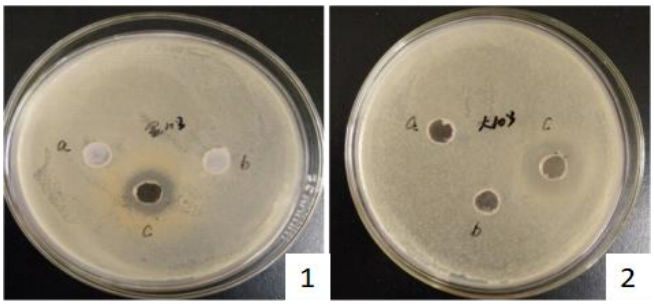


Figure 11. Results of exclude organic acid bacteriostasis test(1 and 2 represent *S. aureus* and *E. coli* for the indicator bacteria antibacterial test, respectively; a and b represent with acetic acid and lactic acid to adjust the pH of the sterile water, respectively, c represents the antibacterial agents)

Identification of the exopolysaccharide antibacterial test

Probiotic exopolysaccharides refer to a class of polysaccharides with long-chain structure secreted by probiotics to the cell surface during growth and metabolism. Some exopolysaccharides also have antibacterial effect. Polysaccharide substances were extracted by ethanol precipitation, deproteinized by protease-Sevag, deproteinized polysaccharide solution was placed on selective semi-permeable membrane for 48 h to remove small molecules. After dialysis, 50 mL of absolute ethanol was added, 4℃ for 24 h, 4000 r/min for 10 min, and the precipitate and supernatant were collected for antibacterial coil test. The results are shown in Figure Figure 12. The isolated sediment had no antibacterial effect, the centrifugal supernatant had obvious antibacterial effect, and the antibacterial effect of the centrifugal supernatant and the untreated S. S. aureus was 17.67 ± 0.14 mm, 19.36 ± 0.18 mm and 17.75 ± 0.20 mm, 19.39 ± 0.13 mm, respectively, so the antibacterial agent produced by this bacterium is not extracellular polysaccharide.

Analysis of the extraction effect of the bacteriostatic agent

After excluding hydrogen peroxide, organic acids and extracellular polysaccharides, the active substances of bacteriostatic agents may be proteins and peptides. Ammonium sulfate precipitation is an effective method for extracting crude proteins, which is easy to operate and can well protect the active of proteins. In the case of unknown proteins, a graded ammonium sulfate salting out was used to determine the optimal ammonium sulfate concentration for the target protein precipitation. The fermentation inhibitors of *L. rhamnosus* L57 grown for 24 h were precipitated with 30%, 40%, 50%, 60%, 50%, 70% and 80% ammonium sulfate for 2 h, centrifuged at 15000 r/min for 30 min, and the precipitate was collected. The precipitate was dissolved with resteamed water. In order to remove the residual ammonium sulfate in the precipitate, cut the 1000Da dialysis bag into 10 cm segments, soaked deionized water for 10 min, washed with deionized water for 3 times, put the dialysis bag with samples into 0.02 mol/L HCL solution for 24 h, and tested the antibacterial activity of the saline supernatant and the precipitated solution, so as to determine the molecular weight of antibacterial substances. The results are as shown in Table 3.

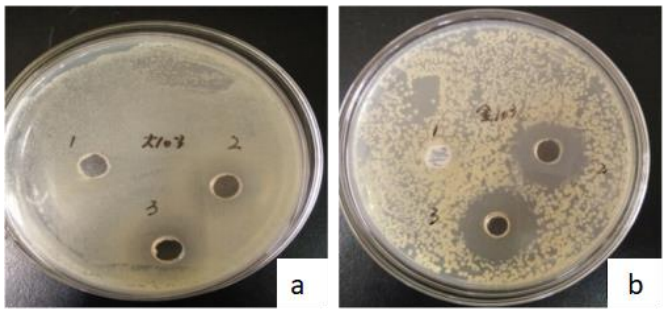


Figure 12. Antibacterial activity of exopolysaccharides (a and b represent the bacteriostasis test of *E. coli* and *S. aureus* as indicator bacteria; 1, 2 and 3 represent the precipitate of the antibacterial agents after ethanol precipitation, the supernatant of centrifugation and the untreated antibacterial agents, respectively)

Table 3. Antibacterial activity of ammonium sulfate salting-out precipitate

Name	Antibacterial activity	Percent of the saturated concentration of ammonium sulfat（%）					
		30	40	50	60	70	80
sediment		+	-	-	+	+	-
The supernatant was centrifuged	*S .aureus*	+	+	+	-	+	+
	E.coli	+	+	+	-	+	+
Precipitation substances	*S .aureus*	-	-	-	+	-	-
	E.coli	-	-	-	+	-	-

Note: + represents a precipitation or an inhibitory circle; -represents no precipitation and no inhibitory circle; E.coli represents E. coli, S.aureus represents Staphylococcus aureus

It can be seen that ammonium sulfate concentration precipitation is 30%, 60% and 70%, 1000Da dialysis bag, after 0.02 mol/L HCl solution dialysis, it was found that only the ammonium sulfate saturation concentration of 60% has antibacterial effect on *E. coli* and *S. aureus*, while the centrifugal supernatant and bacteriostatic agent produced by the bacteria contains protein or peptide substance, the optimal saturation concentration of ammonium sulfate is 60%, the bacteriostasis contains protein or peptide molecular weight> 1000Da.

pH and protease effect on the stability of antibacterial agents in *L. rhamnosus*

E. coli and *S. aureus* were used as indicator bacteria to determine the activity of inhibitor by punching, and the results are shown in Figure 13. At pH 2.0, the bacteriostasis had the strongest activity, and the antibacterial performance of the bacteriostatic agent against the indicator bacteria decreased rapidly, with the antibacterial activity in the pH range of 2.0-4.0, and lost its activity at pH> 5.0.

The results of the analysis of stability inhibitors in *L. rhamnosus* are given in in Figure 14.

Effect of UV on antimicrobial stability of *L. rhamnosus*

The bacteriostatic agent was placed on the ultra-clean workbench and illuminated with 20 W UV lamp under sterile operation, and 6 sets of different irradiation times were set for 0 h, 2 h, 0 h, 4 h, 6 h, 8 h, 10 h, 12 h. After the irradiation, *E. coli* and *Staphylococcus aureus* were measured as the indicator bacteria by agar diffusion punching, and the 0 h inhibitor was used as the control. The results are shown in Figure 15.

The antibacterial activity of the inhibitor with UV lamp irradiation did not change significantly against *E. coli* and *S. aureus*, indicating its excellent stability under UV light.

Investigation of the thermal stability of *L. rhamnosus* inhibitor

The bacteriostatic agents were treated at 4℃, 30℃, 40℃, 50℃, 60℃, 70℃, 90℃, 100℃, and 120℃ respectively for 30 min, and the antibacterial agent activities were determined by using *E. coli* and *S. aureus* as the indicator bacteria. The results were shown in Figure 16.

The antibacterial activity of the inhibitor changed little after treatment at different temperatures, indicating the good thermal stability of the inhibitor produced by *L. rhamnosus* L57.

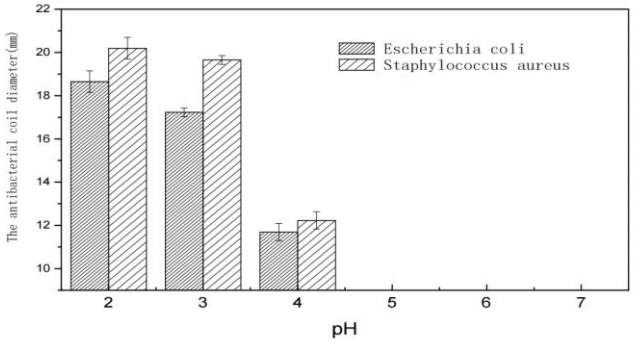


Figure 13. Effect of pH on activity of antibacterial agents

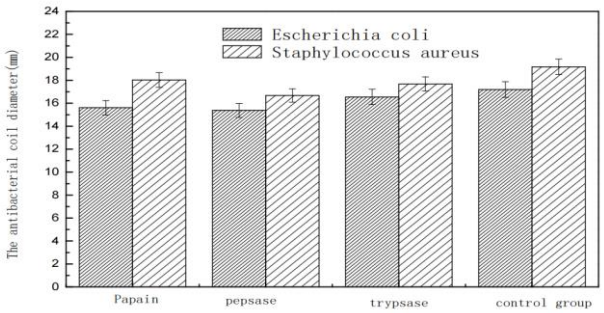


Figure 14 Effect of protease on activity of antibacterial agents

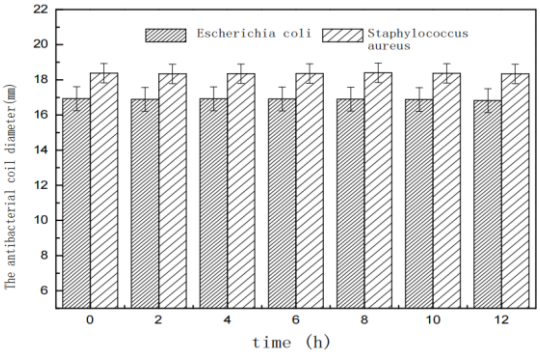


Figure.15 Effect of UV on activity of antibacterial agents

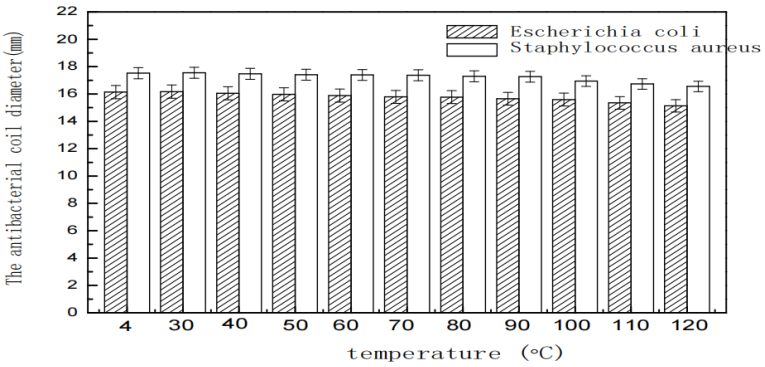


Figure 16. Effect of temperature on activity of antibacterial agents

Application in the preservation of bananas of Antibacterial agents from *L. rhamnosus* L57 with natamycin and chitosan

Changes in the acidity of bananas: The changes in acidity of bananas treated with different preservatives during storage are shown in Figure 17. The acidity of bananas increased with the prolonging of storage days. Compared with the untreated control group, the acidity of bananas treated with antibacterial agents in the C+N+2PAA group changed more obviously, and the acidity of bananas treated with antibacterial agents in the C+N+2PAA group changed less, namely 1.5% chitosan 1.25% bacteriostatic lyophilized powder and 0.03% natamycin could effectively slow down the acidity change of banana, but could not completely inhibit the ripening of banana.

Change of soluble solids in banana: Banana in the process of preservation soluble solids (sugar is the main soluble solids) is increasing; change as shown in Figure 18. After different preservative treatment, changes in soluble solids is different; with the increase of storage days, total soluble solids are also on the increase, due to the transformation during storage of banana pulp starch into sugar. Compared with the other groups, antistaling agent C+N and C+N+2PPA group shows not significant change. Chitosan is producing bacteriostatic agent and can delay the banana maturation; this is because the antistaling agent can inhibit the fruit respiration, lowering the enzyme activity and inhibiting the production of metabolites, reduce the rate at which carbohydrates hydrolyzed into sugar.

Banana sensory score by the degree of Browning of the banana: as shown in Figure 19, the degree of browning and wilting degree is of important indexes of banana freshness degree, which reduce with the increase of storage days' sensory score. Different antistaling agent show differences in sensory score; antistaling agent C+N and C+N+2PAA group has the highest sensory score. After 12 days of storage, the control group had lost its edible value, while the other groups still had edible value, indicating that the use of preservative treatment can improve the sensory quality of bananas.

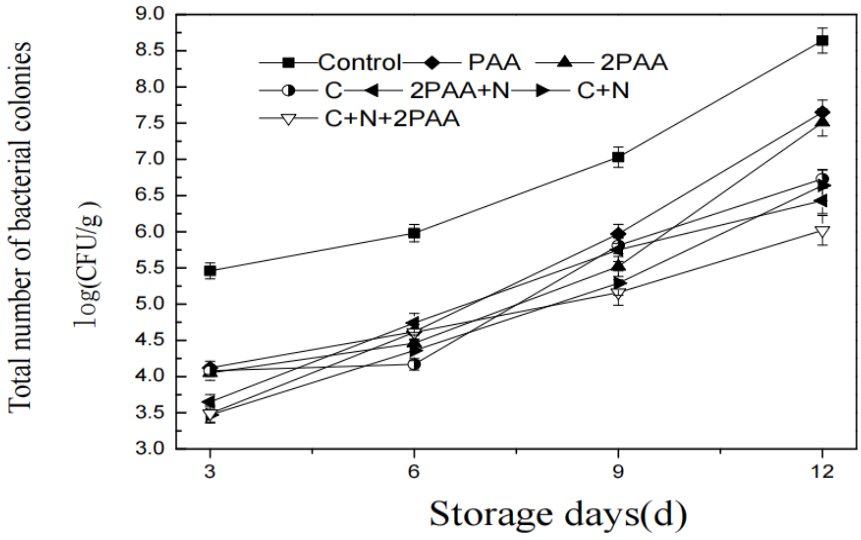


Figure 17. Effect of different preservatives on acidity of banana

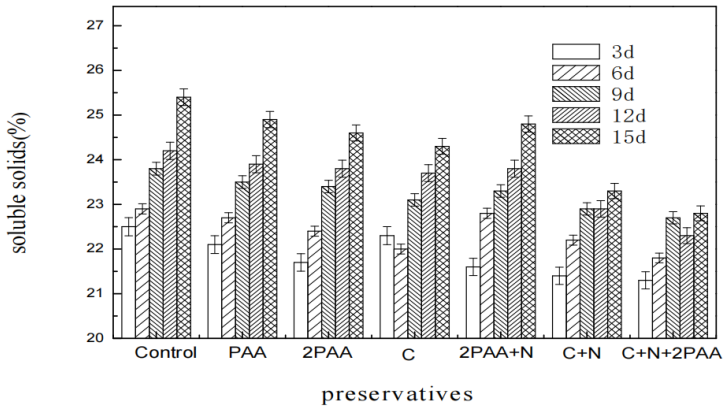


Figure 18. Effect of different preservatives on soluble solids of banana

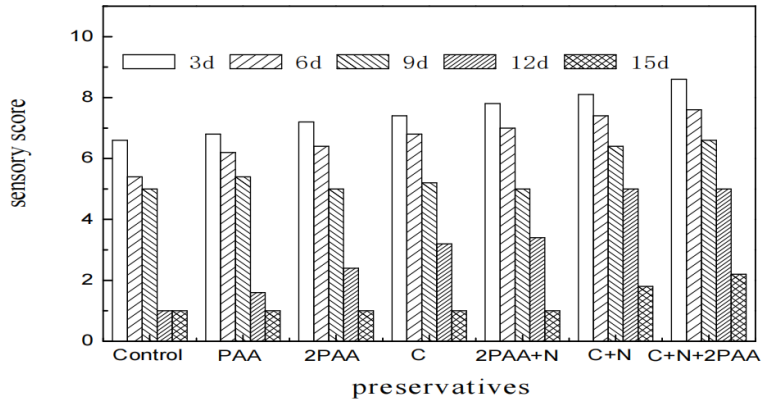


Figure 19. The results of sensory score of banana

Banana appearance change in the process of storage is shown in Figure 20; groups of antistaling agent can delay the banana appearance color change. Appearance change of PAA and PAA2 group were similar, then any other group of preservation effect is poorer. The appearance of the control group had a significant difference, the control surface of banana changed. The speed was faster, the surface of the preservative treatment was relatively bright, the surface part appeared black spots and wilting and the speed of change was slow. C+N+2PAA group had the best effect, followed by C+N group.

Shelf life survey of bananas: Twenty-one bananas at green ripening stage were randomly divided into 7 groups with 3 bananas in each group. After that, different preservatives were used to treat the bananas, and the untreated group was used as a control. The shelf life was calculated by the time the bananas were stored at room temperature to maintain optimal marketing and edible quality. Table 4 lists the results; the shelf life of bananas in the control group was the shortest, 10.8 days. The shelf life of bananas in C+N+2PAA group was the longest, 14.5 days. It can be seen that the preservative can prolong the shelf life of bananas, and the effect of chitosan, natamycin and preservative lyophilized powder is the best. According to the experiments of PAA and 2PAA group, the application of bacteriostatic freeze-dried powder alone extended the shelf life of banana to a certain extent, but the effect was not obvious. Bacteriostatic freeze-dried powder could inhibit the decay caused by surface microorganisms of banana, but could not completely inhibit the ripening of banana.

Analysis of weight loss change of banana: The weight loss changes of bananas treated with different preservative during storage are shown in Figure 21. The weight loss changes of the control group are significantly higher than those of the other groups, the weight loss changes of the preservative C+N+2PAA group are the least, and the weight loss changes of the preservative C C+N and C+N+2PAA groups are significantly lower than those of the other groups, while the preservative PAA group is significantly lower. The weight loss of 2PAA and 2PAA+N groups was slightly slower than that of the control group, but significantly higher than that of the preservative C C+N and C+N+2PAA groups. Chitosan may form a high molecular protective film on the surface of banana to prevent the transport of nutrients and the loss of water, reduce the respiration rate and the diffusion of water, while the bacteriostatic lyophilized powder and natamycin have no such effect.

Table 4. Effect of different preservatives on the shelf life of bananas

Group preservatives	shelf life/d
Control	10.8±0.8
PAA	11.6±0.6
2PAA	11.7±0.65
C	12.6±0.55
2PAA+N	12.4±0.8
C+N	13.7±0.95
C+N+2PAA	14.5±1.2

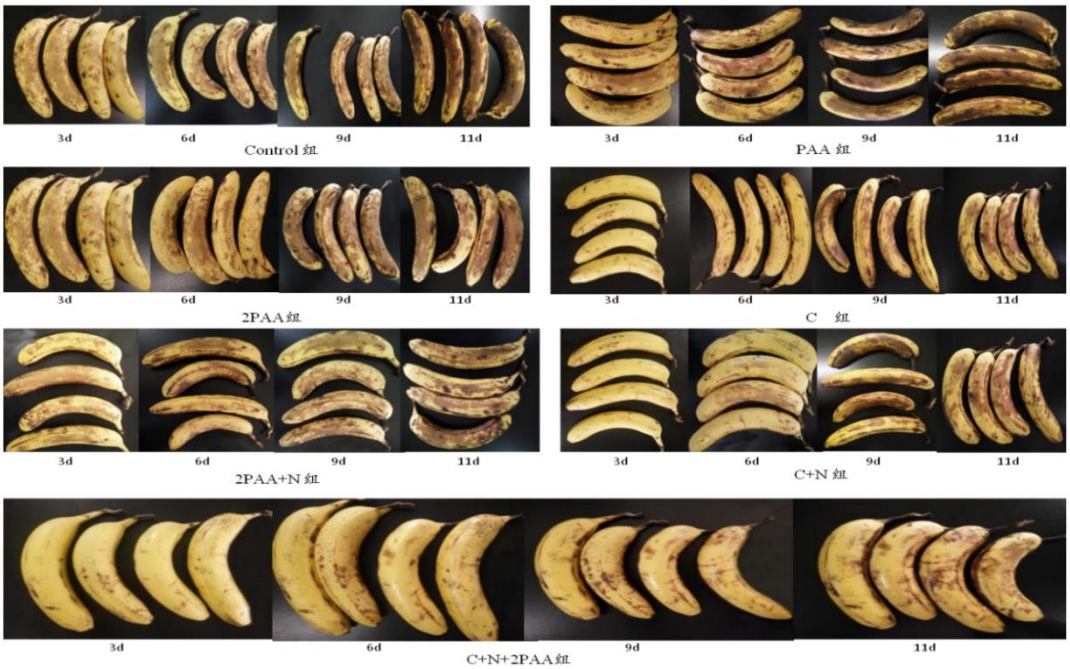


Figure 20. Effect of different preservatives on the changes appearance of banana

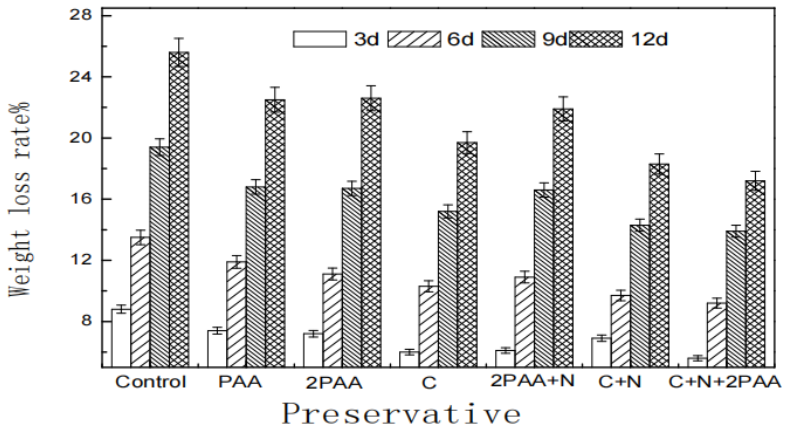


Figure 21. Efect of different preservatives on the weight loss rate of bananas

Analysis of banana color change: The color change of bananas is an important indicator for consumers to judge the freshness. The color difference change results of bananas treated with different preservatives during storage are shown in Table 5. L* reflects the brightness of bananas, and a higher value of L* indicates a better brightness of bananas. A * indicates the redness of bananas. The larger the value, the redder the bananas will be. B * reflects the yellowness of the banana, and the higher the value, the yellower the banana. The brightness and yellowness of bananas decreased with the increase of storage time, while the redness also showed a decreasing trend but was not obvious. The

brightness and yellowness of bananas decreased more at 3-6 days of storage than at other time periods, and the Browning of bananas was obvious at 12 days of storage. The L* and B * values of bananas in the control group were 24.38 and 10.86, respectively. However, the maximum L* and B * values of group C+N+2PAA were 42.73 and 22.66, respectively. Therefore, the combination of group C+N+2PAA could slow down the change rate of brightness and yellowness of bananas.

Changes in total microbial population of banana: Bananas are often decomposed by disease after harvest, and microorganisms are the main cause of disease. Reasonable control of microbes was beneficial to reduce the incidence of banana, and the changes in the total number of colonies from different treatments are shown in Figures 22 and 23. The total number of banana colonies in storage rose has a varying degree (Figure 23); the control group rose relatively fast, compared with the control group can inhibit bacteria and mold, and PAA and 2PAA on mold is slightly lower than the control group, this is the antibacterial powder on the bacteria, preservative C + N + 2PAA group of bacteria and mold are lower than other group, so the preservative can inhibit the bacteria and mold on the surface of banana.

Table 5. Effect of different preservatives on colour (score) of banana

DAY/GROUP		3d	6d	9d	12d
Control	L*	58.61±0.68	36.00±1.28	31.61±1.25	24.38±0.65
	a*	12.26±1.23	10.52±1.08	8.36±0.82	8.63±0.82
	b*	48.16±0.98	22.68±0.92	12.54±1.24	10.86±0.55
PAA	L*	59.34±1.44	37.12±0.44	35.34±1.03	28.92±0.76
	a*	10.79±1.02	9.74±1.27	8.52±1.33	8.94±0.88
	b*	49.42±0.87	24.66±0.77	16.39±1.41	12.91±0.94
2PAA	L*	60.11±1.12	38.73±1.43	36.33±1.11	31.29±0.66
	a*	10.79±0.99	9.82±0.78	8.63±0.45	8.84±0.84
	b*	49.42±1.56	26.60±1.16	17.11±0.54	13.34±0.92
C	L*	60.53±1.65	43.63±1.04	39.63±1.05	38.33±0.73
	a*	11.24±1.35	10.42±1.26	9.83±1.03	10.25±0.84
	b*	49.94±1.21	26.60±0.86	19.21±0.66	17.95±1.04
2PAA+N	L*	60.23±0.86	34.78±0.65	38.12±0.77	35.37±0.46
	a*	10.99±1.54	9.04±1.13	9.24±1.01	8.47±0.71
	b*	49.51±1.27	18.07±1.36	18.32±0.75	13.33±0.93
C+N	L*	62.39±1.64	46.45±1.03	40.33±0.78	38.51±0.65
	a*	11.26±1.05	11.54±1.14	9.76±0.65	10.26±1.02
	b*	51.37±0.93	24.03±0.82	19.07±0.92	18.01±0.87
C+2PAA + N	L*	63.74±1.18	47.86±1.31	44.41±0.89	42.73±0.38
	a*	11.54±1.32	11.18±1.02	10.74±1.01	9.82±0.44
	b*	53.83±0.76	31.57±0.86	25.05±1.12	22.66±0.53

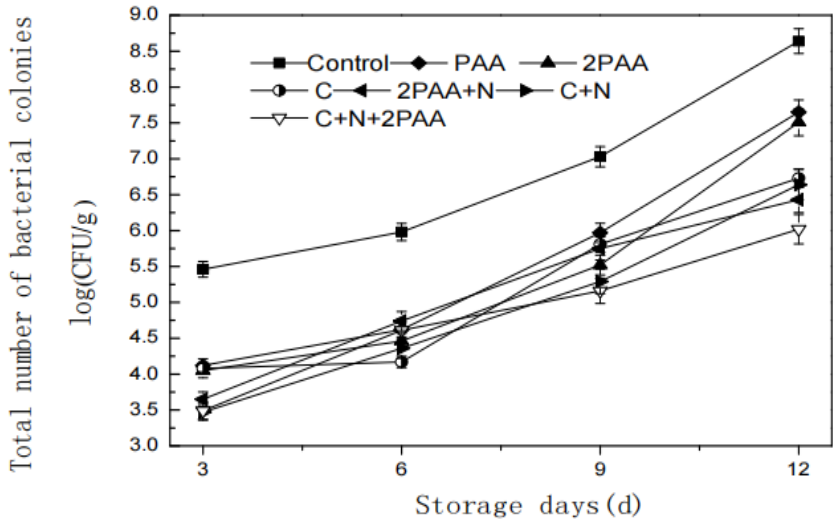


Figure 22. Changes of the total number of bacterial of banana at different storage times

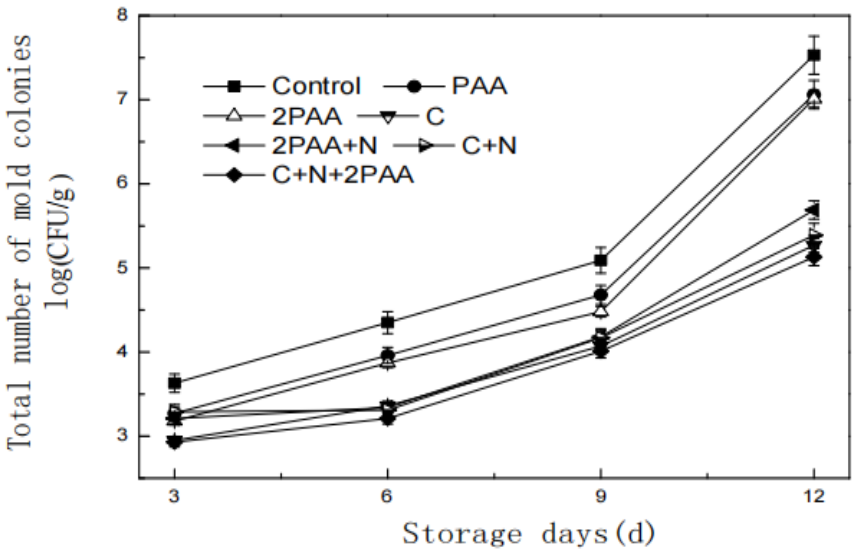


Figure 23. Changes of the total number of mold of banana at different storage times

DISCUSSION

Banana rot is a natural process that occurs due to various reasons such as harmful microorganisms, enzyme decomposition in tissues, mechanical damage during picking and transportation, and environmental conditions like temperature, humidity, air, and light. Microorganisms are the primary factors leading to it corruption (Sebranek et, al, 2005). In the presence of microorganisms, banana and its protein will be decomposed and used, resulting in sour and smelly banana, loss of original toughness and elasticity, and darker color. Banana also contains various enzymes that can decompose nutrients in banana and cause banana corruption. In this topic, the shelf life of the banana was extended to 4d, thus inhibiting the growth of harmful microorganisms.

Chitosan is a polysaccharide widely used in food preservation as a natural preservative. Chitosan can form a protective film on the surface, isolating oxygen from the outside world, and reducing respiratory intensity. Chitosan has a preservation effect, and its antibacterial mechanism is believed to be chitosan adsorption on the cell surface to form a layer of a polymer membrane that prevents nutrient transport between cells and has an antibacterial effect. Chitosan can penetrate into the cell body, adsorb cells with anion cytoplasm and flocculation, thus disturbing the normal physiological activities of cells and having an antibacterial effect (Muzzarelli et, al, 1990).

Natamycin is a colorless, odorless, low dose and high safety of food additives that mainly inhibits fungi (Yuan et, al, 2022), bacteriostatic lyophilized powder mainly antibacterial to bacteria, it may act on the cell membrane and block bacterial cell wall peptidoglycan biosynthesis. It can also inhibit the synthesis of cell membrane and phospholipid compounds. It may also use ion interaction to form membrane pores that lead to cellular material outflow and cell deat (Anjana et, al, 2022).

For the use of chitosan combined with natural preservatives for fruits and vegetables and fresh meat, the have found that chitosan has some effect on extending the shelf life of cod (Skírnisdóttir et, al, 2020). Other studies show that by using chitosan to treat sugarcane, the shelf life of sugarcane, banana, Longyan and other are prolonged (Karamchandani et, al, 2022; Martelli et, al, 2013), which is expected to become an anti-corrosion material for food packaging.

Using chitosan combined with plastic wrap to keep bananas fresh, it was found that as the storage time was extended by more than 5 days. In addition, when the concentration of chitosan solution is 1%, the preservation effect of bananas is the best (Fu et, al, 2021). Fresh film with 2% chitosan solution was found to prolong the shelf life and prevent after 21 days of storage (Hoa et, al, 2022). Starch aldehyde-catechin and chitosan cooperate with lactic acid to preserve the fresh meat. The results show that starch aldehyde-catechin and chitosan work together, respectively. Edible coatings containing antioxidants and antibacterial agents can form water and oxygen barriers around pork, and inhibit the growth of oxidation and microorganisms in pork, thus playing a role in preservation.

CONCLUSION

Twenty-three probiotic strains were screened using *E. coli*, *S. aureus*, *P. chrysogenum*, and *V. alginolyticus* as indicator microorganisms, and the inhibitory effect of the L57 strain was identified and determined. The strain was identified as *L. rhamnosus* by molecular means through gene extraction and PCR amplification product comparison with the NCBI database. The antibacterial agent lyophilized powder and biological antibacterial agent alone or compound preparation compound preservative were used for banana preservation. The results showed that compound group preservation was better for the mixture containg chitosan 1.5% + antibacterial agent lyophilized powder +1.25%, and natamycin 0.03%. It can slow the ripening of bananas and prevent corruption, extending the shelf life by 4 days.

The combination of broad-spectrum and safe probiotics antibacterial agent freeze-dried powder prepared in this study with other preservatives can significantly prolong the storage period of fruits and vegetables, reducing rot and waste.

ACKNOWLEDGMENTS

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REFERENCES

1. Abubakr, M., & Aladiwish, W. (2017). Isolation and identification of lactic acid bacteria from different fruits with proteolytic activity. *Int. J. Microbiol. Biotechnol.* 2(2), 58–64. DOI:10.11648/j.ijmb.20170202.12.
2. Anjana,T., Santosh, K. (2022). Bacteriocin-Producing Probiotic Lactic Acid Bacteria in Controlling Dysbiosis of the Gut Microbiota.*Front. Cell Infect. Microbiol.*12:851140.DOI：10.3389/fcimb.2022.851140.
3. Du, G., Chang, S., Guo, Q. (2022). Protective effects of Tibetan kefir in mice with ochratoxin A-induced cecal injury.*Food Res. Int.* 158: 111551. DOI: 10.1016/j.foodres.2022.111551.
4. Deegan, L ., Cotter, P., Hill, C., et al. (2006).Bacteriocins: biological tools for bio-preservation and shelf-life extension. Int. Dairy J. 16(9):1058-1071. DOI: 10.1016/j.idairyj.2005.10.026.
5. Fu, B.,Mei, S.,Su, X. (2021). Integrating waste fish scale-derived gelatin and chitosan into edible nanocomposite film for perishable fruits. *Int. J. Biol. Macromol.* 191:1164-1174. DOI:10.1016/j.ijbiomac.2021.09.171.
6. García, P, Rodríguez, L, Rodríguez, A. (2010). Food biopreservation: promising strategies using bacteriocins, bacteriophages and endolysins. *Trends Food Sci. Technol*. 21(8):373-382. DOI:10.1016/j.tifs.2010.04.010.
7. Grace, Zhang. (2013). Foodborne Pathogenic Bacteria Detection: An Evaluation of Current and Developing Methods. *Meducator,* 1(24):27-30. DOI:10.15173/m.v1i24.835.
8. Gulahmadov, S., Abdullaeva, N., Guseinova, N. (2009).Isolation and characterization of bacteriocin-like inhibitory substances from lactic acid bacteria isolated from Azerbaijan cheeses. *Appl. Biochem. Microbiol*. 45(3):266-271. DOI: 10.1134/S0003683809030053.
9. Hugo, C., Hugo, A. (2015). Current trends in natural preservatives for fresh sausage products. *Trends Food Sci. Technol*. 45(1):12-23. DOI: 10.1016/j.tifs.2015.05.003.
10. Hoa, Van-Ba., Song, Dong-Heon., Seol, Kuk-Hwan., Kang, Sun-Moon., Kim, Hyun-Wook., Kim, Jin-Hyoung., Moon, Sung-Sil., Cho, Soo-Hyun. (2022). Application of a Newly Developed Chitosan/Oleic Acid Edible Coating for Extending Shelf-Life of Fresh Pork. *Foods*,11(13),1978. DOI:10.3390/foods11131978.
11. Hossain, M. (2013). Extraction and characterization of chitosan from shrimp shell and its utilization to increease shelf of banana. *Biochem. J*.187(1):45-51. DOI:10.1042/bj1870045
12. Kubo, Y, Inaba, A, Nakamura, R. (1990). Respiration and C_2H_4 production in various harvested crops held in CO_2-enriched atmospheres. *J. Am. Soc. Hortic. Sci.*115(6):975-978. DOI:10.21273/JASHS.115.6.975.
13. Karamchandani, B.,Chakraborty, S., Dalvi S. (2022). Chitosan and its derivatives: Promising biomaterial in averting fungal diseases of sugarcane and other crops. *J Basic Microbiol.*62: 533-554.DOI: 10.1002/jobm.202100613

14. Lone, G., Lars R., Maria R. (2002). Food spoilage-interactions between food spoilage bacteria. *Int. J. Food Microbiol.* 78(1-2):79-97. DOI:10.1016/S0168-1605(02)00233-7.
15. Leroy, F, Lde V. (2004).Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci. Technol*. 15(2):67-78. DOI: 10.1016/j.tifs.2003.09.004.
16. Leroy, F, Vuyst, L. (2005). Bacteriocin-producing strains in a meat environment. *Methods in biotechnology*, 18:369-380. DOI: 10.1385/1-59259-847-1:369.
17. Muzzarelli, R, Tarsi, R, Filippini, O.(1990). Antimicrobial properties of N-carboxybutyl chitosan. *Antimicrob. Agents Chemother*. 1990, 34(10):2019-2023. DOI:10.1128/aac.34.10.2019.
18. Michaylova, M.,Yungareva, T.,Urshev, Z. (2021). Probiotic candidates among dairy *Lactobacilli* and *Streptococcus thermophiles* strains for control of the oral pathogen *Porphyromonas gingivalis*. *Folia Med. (Plovdiv)*. 63: 720-725. DOI: 10.3897/folmed.63.e56551.
19. Martelli, M., Barros, T., de Moura, M., Mattoso, L., Assis, O. (2013). Effect of chitosan nanoparticles and pectin content on mechanical properties and water vapor permeability of banana puree films. *J. Food Sci.* 78(1), 98-104. DOI:10.1111/j.1750-3841.2012.03006.x
20. Nisia, C., Caciano, P., Noreña, Adriano, B. (2012). Antimicrobial activity of chitosan films containing nisin, peptide P34, and natamycin. Cyta J.Food, 10(1): 21-26. DOI:10.1080/19476337.2010.537371.
21. O'Brien, S. (2012). The public health impact of food-related illness. Current opinion in infectious diseases. *Curr. Opin. Infect. Dis.* 25(5):537-45. DOI:10.1097/QCO.0b013e328356aeba.
22. Poveda, J., Loarce L., Alarcón, M.(2018). Revalorization of winery by-products as source of natural preservatives obtained by means of green extraction techniques. *Ind. Crops Prod.* 112:617-625. DOI: 10.1016/j.indcrop.2017.12.063.
23. Scallan, E., Hoekstra, R., Angulo, F. (2011). Foodborne ill-nessacquiredin the United States-major pthogens. *Emerg. Infect*. Dis. 17(1):7-15.DOI:10.3201/eid1701.091101p2.
24. Sebranek, J., Sewalt, V., Robbins, K. (2005).Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Sci.* 2005, 69(2):289-296. DOI: 10.1016/j.meatsci. 2004.07.010
25. Skirnisdottir, S., Knobloch, S., Lauzon, H. (2021). Impact of onboard chitosan treatment of whole cod (Gadus morhua) on the shelf life and spoilage bacteria of loins stored superchilled under different atmospheres.*Food Microbiol*. 97: 103723.DOI:10.1016/j.fm.2020.103723.
26. Velusamy, V., Arshak, K., Korostynska, O. (2009).Anover-view of foodborne pathogen detection: in the perspective of biosensors.*Biotechnol. Adv.* 28(2):232-254. DOI: 10.1016/j.biotechadv.2009.12.004.
27. WHO, FAO. (2001) Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Amerian córdoba park hotel córdoba argentina: WHO&FAO, 2001.
28. Woods, L. & Church, P. (1999). Strategies for extending the Shelf-life of poultry meat and products.In, Richardson R. & Mead, G. P*oultry Meat Science*, (pp. 297-300). Wallingford: CABI Publishing.
29. Holzapfel, W., Geisen, R., Schillinger, U. (1995).Preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *Int. J. Food Microbiol.* (24):343-362. DOI:10.1016/0168-1605(94)00036-6
30. Yuan, Y., Wang, H., Fu, Y. (2022).Sodium alginate/gum arabic/glycerol multicomponent edible films loaded with natamycin: Study on physicochemical, antibacterial, and sweet potatoes preservation properties.*Int. J. Biol. Macromol.* 213: 1068-1077.DOI:10.1016/j.ijbiomac.2022.06.040.