

Study on Isolation and Identification of *Lactobacillus plantarum* DC-3 in Pickled Cucumber

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Abstract: This experiment aims to explore the diverse beneficial effects of *Lactobacillus plantarum*. Employing the dilution coating method, eighteen strains of *Lactobacillus* were isolated and screened from commercially available pickled cucumbers. Subsequent evaluations were carried out to assess their probiotic potential, including examinations of their gastrointestinal tolerance and antioxidant activity. Through these analyses, a highly adaptable strain was identified. The results demonstrate that the strain designated as DC - 3 exhibits remarkable cholesterol - lowering capabilities. Moreover, it sustains a survival rate exceeding 70% in bovine bile salt media with concentrations ranging from 0.3% to 1%, even within the gastrointestinal environment, where the tolerance level can reach up to 75%. The cell's capacity to scavenge DPPH radicals and hydroxyl radicals is 37.73% and 46.71%, respectively. Additionally, the self - aggregation and co - aggregation abilities are 64% and 28.5%, respectively. These findings suggest that *Lactobacillus* DC - 3 presents potential beneficial effects and provides valuable reference information for the future development of *L. plantarum* - related fermented food.

Keywords: Cucumber; *Lactobacillus*; Cholesterol; Probiotics; Antioxidant activity

1. Introduction

1.1 Introduction to Pickled Cucumber

Pickled cucumber is a type of pickled vegetable, typically produced using stem cucumbers as the primary raw material [1]. The processing involves extracting water from the vegetable, thus giving it the name "pickled cucumber."

After processing and curing, pickled cucumber has become a prevalent flavor in everyday cuisine. Wujiang cucumber is the most renowned variety, recognized as the foremost among the world's three famous pickles and consistently regarded as a superior vegetarian dish. Its distinctiveness lies in its unique production techniques and refined ingredients. There is a significant amount of *Lactobacillus* in pickled cucumber, and in the early stage of fermentation, the number of mixed bacteria on the surface is greater than that of *Lactobacillus*. With the progress of fermentation, the variety and number of miscellaneous bacteria decrease, while the number of *Lactobacillus* increases rapidly. At the later stage of fermentation, the microorganisms in the pickled cucumber are almost all *Lactobacillus* [2]. In 1930, Pederson first proposed that *Leuconostoc* plays a role in initiating the lactic acid fermentation of vegetables. Modern nutrition believes that pickled cucumber can strengthen the spleen and stimulate the appetite, replenish qi and essence, increase food intake, and help invigorate the spirit. Low - salt pickled cucumber also has the effects of protecting the liver and reducing weight. Pickled cucumber is said to have a "natural halo haining" effect, and chewing a piece of pickled cucumber in the mouth can effectively relieve symptoms such as motion sickness. One can also eat pickled cucumber to relieve dizziness when drinking too much alcohol. In recent years, Chinese scholars have conducted extensive research on the fermentation process of pickles, but relatively little research has been done on *Lactobacillus* in pickles. Compared with the research on microorganisms in pickles, it can play a decisive role in the development of the pickles industry, so our research on pickles is more meaningful.

1.2 Overview of *L. plantarum*

Lactobacillus is the general term for a class of spore - free, gram - staining positive bacteria whose main product of fermented sugars is lactic acid. The physiological function of *L. plantarum* is rich [3], and it can grow at 15°C, with the optimal growth temperature being 30 - 35°C. It is a type of chemoheterotrophic microorganism, and the strain is straight or curved rod - shaped, single, paired, or in chains. The most suitable pH is around 6.5, and it belongs to the homofermentative *Lactobacillus*. *L. plantarum* is a probiotic found in the human gastrointestinal tract. It can metabolize various natural antibacterial substances such as organic acids, bacteriocin, hydrogen peroxide, and butanedione, which play a role in promoting human health [4]. *L. plantarum* has been widely used in the screening of probiotics due to its safe source. It has strong tolerance to the high acidity and high salt environment of fermented foods such as cucumber, olive, sauerkraut, and fermented dairy products, and is thus widely used in the food industry [5]. In recent years, with the in - depth study of *L. plantarum*, it has been found that many types of *Lactobacillus* have probiotic effects.

1.3 Physiological Function of *L. plantarum*

L. plantarum exists in the human intestinal tract and must reach the small intestine and large intestine in a viable state through the acidic environment of the stomach and the bile salt environment of the duodenum to play a role in regulating the flora [6]. Therefore, we can utilize the various properties of *Lactobacillus* for our benefit, and the study of its physical and chemical properties is more meaningful. Cholesterol is an essential substance in the body, not only involved in the formation of cell membranes but also serving as a raw material for the synthesis of bile, vitamin D, and steroid hormones. However, a high level of cholesterol in the human body may lead to cardiovascular diseases, which are unfavorable to human health. The idea that *Lactobacillus* can degrade cholesterol was first proposed by Gililand S et al. in 1985. *Lactobacillus* also has a certain tolerance to gastrointestinal conditions. It can form a physiological barrier on the gastrointestinal lining, accelerate the digestion and absorption of food, and also inhibit the growth of pathogenic bacteria. Currently, people's research on *Lactobacillus* is becoming

increasingly in - depth, and its probiotic functions such as improving intestinal environmental function, preventing lactose intolerance, promoting the absorption of nutrients, inhibiting the absorption of cholesterol, lowering blood lipids and blood pressure, protecting the liver, and enhancing the detoxification of the liver [7-12] have gradually received attention.

1.4 Development and Utilization of *L. plantarum*

Intestinal microecological balance is crucial for maintaining intestinal health and the productivity of animals. The quantitative ratio of *Lactobacillus* to *Escherichia coli* is considered an important index for evaluating the microecology of the digestive tract, and a higher quantity of *Lactobacillus* than *Escherichia coli* can promote the inhibition of beneficial bacteria on intestinal pathogenic bacteria. Adding *Lactobacillus* to dairy products can produce yogurt, cheese, and other foods, endowing dairy products with a unique flavor. Using *Lactobacillus* to ferment fruit and vegetable products can not only enhance the flavor and nutritional value of fruit and vegetable products but also prevent the growth of spoilage bacteria and enhance their health care function. *Lactobacillus* can also be applied to the production of meat products [13], which can significantly improve the appearance of meat products, promote the improvement of taste, and reduce the generation of nitrite, thereby greatly improving the quality of meat products. *Lactobacillus* is also widely used in various functional foods due to its probiotic effect and special physiological effects.

1.5 The Purpose and Significance of the Study

With the improvement of people's living standards, there are increasingly higher requirements for health, which implies more profound requirements for food culture. Since ancient times, China has emphasized the homology of medicine and food, and there is no absolute dividing line between them. *Lactobacillus* has many probiotic effects, but there is still much room to explore how these effects can be utilized by us. Therefore, it is necessary to select *L. plantarum* that can effectively degrade cholesterol and has a high survival rate in high - concentration bile salt and the gastrointestinal tract, so that it can smoothly

enter the gastrointestinal tract and exert its biological effect, and further study its probiotic effect. This lays a foundation for the better development and utilization of *Lactobacillus* in the future.

2. Materials and Methods

2.1 Principal Reagent

MRS Broth culture-medium, AGAR, Calcium carbonate, Twain, Cholesterol, bovine bile salt, anhydrous ethanol, phenanthroline, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, ferrous sulfate, concentrated sulfuric acid, Columbia Blood AGAR medium, hydrogen peroxide.

2.2 Instrument and Equipment

Ultra-clean workbench, biochemical incubator, constant temperature culture concussion tank, high pressure steam sterilization pot, constant temperature water bath electronic balance, ultraviolet spectrophotometer, microwave oven, volumetric bottle, beaker, glass rod, conical bottle, inoculation ring, disposable gloves, pipette gun.

2.3 Experimental Sample

Brand - packaged pickled cucumbers were collected from the department store and brought back to the laboratory, and the oily and bright cucumbers without deterioration and corruption were selected as the experimental samples.

2.4 Experiment with Relevant Solution Formulations

MRS Medium: Add AGAR (1.5g/100mL) to MRS Solid medium (5.224g/100mL), heat to boiling in microwave oven, then add calcium carbonate (1.5g/100mL) and stir.

MRS Broth medium: Add 1.5g/100mL calcium carbonate into MRS (5.224g/100mL) and stir.

Cholesterol-MRS medium: Heat and dissolve 2% bovine bile salt, 1g/L cholesterol, then mix the prepared cholesterol - bovine bile salt solution with MRS Broth medium, and fix the volume to 1L for use.

DPPH reserve liquid: Dissolve 20mg DPPH standard to 10mL anhydrous ethanol and store at -20°C (not suitable for long-term storage).

DPPH working liquid: Take 1 mL DPPH reserve liquid and place it in a 100 mL brown volumetric bottle with a concentration of 0.02 mmol/L (ready for use).

PBS phosphate buffer: Add 45.644g and 27.22 g potassium dihydrogen phosphate to 1L of water, respectively.

OPA reagent formulation: Weigh 0.05g of solid phthalic formaldehyde, dissolve it with ice acetic acid to 100mL in the fume hood, and prepare it for use.

Artificial stomach acid formula: Pepsin (1:500)3.5g/L, NaCl2g/L, adjusted to pH = 3 with hydrochloric acid. Formula: trypsin (1:250)1g/L, bile salt 1.2 g/L, NaCl 4.4g/L, NaHCO₃ 2.2g/L, adjusted to pH=8 with NaOH. Gastrointestinal environment: A Mix of the above two.

2.5 Isolation and Screening of *Lactobacillus*

The samples of 10g pickled cucumber were put into a sterile mortar for grinding, and the ground crushed pickled cucumber and juice were put into 50mL sterile water. Then the mixed solution was diluted in a gradient, and the samples with different dilution ratios were inoculated into MRS Broth medium and incubated at 37°C for 48h. The single colony with a large transparent circle was selected for plate scribbling culture, and after 3 generations of culture, a catalase test was carried out. The strain with a negative test result was inoculated into MRS Broth medium for constant temperature cultivation at 37°C for 48h and stored in a tube at 4°C for future use.

2.6 The Action of *Lactobacillus* to Degrade Cholesterol

2.6.1 Qualitative determination of cholesterol degrading ability

Lactobacillus liquid cultured for 48h was mixed with cholesterol-MRS liquid broth medium at the ratio of 1:19. After culture at 37°C for 24h, the cholesterol content of *Lactobacillus* was measured by the phthalaldehyde method. Add 1mL sample, 2mL 50% KOH solution, and 3mL anhydrous ethanol into the test tube and place them in water at 60°C for 0.5h. Cool to room temperature first, then add 3mL n-hexane and fully shock, and finally add 3mL distilled water. Wait for stratification, absorb the upper liquid, and dry at 60°C. Add 2mL OPA reagent to the dried sample, and add 2mL concentrated sulfuric acid after reacting at room temperature for 10min. After fully shaking for 20s, measure the absorbance at 550nm wavelength.

2.6.2 Detection of BSH enzyme activity

The filter paper sheet made by the perforator was put into the sterilizing pot for sterilization,

and the plate was prepared in advance. First, add 0.5% sodium taurine deoxycholic acid and 0.37g/L CaCl₂ to the MRS Broth medium, then add 5μL of the overnight culture supernatant, and observe the filter paper after culturing at 37°C for 72h without oxygen. If BSH is positive, white precipitating rings will be found around the filter paper sheet. If BSH is negative, there will be none. First, add 0.5% sodium taurine deoxycholic acid and 0.37g/L CaCl₂ to the MRS Broth medium, then add 5μL of the overnight culture supernatant, and observe the filter paper after culturing at 37°C for 72h without oxygen. If BSH is positive, white precipitating rings will be found around the filter paper sheet. If BSH is negative, there will be none [14].

2.7 Antioxidant Activity

2.7.1 Determination of DPPH free radical scavenging ability

Take a 0.2 mL sample, add 2 mL of DPPH working liquid, and react in the dark for 0.5h. Centrifuge at 6000rpm for 10min, and then measure the absorbance at 517nm wavelength and record it as A₀. Replace the sample with anhydrous ethanol and repeat the above operation, and the measured value is A₁. Replace DPPH with anhydrous ethanol and record it as A_i. Use Vc to make a standard curve. The DPPH free radical clearance rate of the sample to be tested is converted to Vc equivalent, and then the DPPH free radical clearance rate is calculated as shown in equation (1).

$$\text{DPPH free radical clearance} = [1 - (A_0 - A_1)/A_i] \times 100\% \quad (1)$$

2.7.2 Determination of hydroxyl radical scavenging ability

Fully react 1 mL of 2.5 mmol/L phenanthroline, 1mL of 0.02 mmol/L PBS phosphate buffer, 1 mL of distilled water, and 1 mL of 2.5 mmol/L ferrous sulfate, and then add 1 mL of 20 mmol/L hydrogen peroxide solution. Measure the absorbance at 536nm after reacting at room temperature for 1.5h and record it as AP. If the hydrogen peroxide solution is replaced with distilled water, record it as AB. Distilled water is recorded as A_s, and Vc is used as a positive control. The hydroxyl free radical clearance rate of the sample to be tested is converted to Vc equivalent, and the hydroxyl free radical clearance rate is calculated in equation (2). Fully react 1 mL of 2.5 mmol/L phenanthroline, 1mL of 0.02 mmol/L PBS phosphate buffer, 1 mL of distilled water, and 1 mL of 2.5 mmol/L ferrous sulfate, and then add 1 mL of 20 mmol/L

hydrogen peroxide solution. Measure the absorbance at 536nm after reacting at room temperature for 1.5h and record it as AP. If the hydrogen peroxide solution is replaced with distilled water, record it as AB. Distilled water is recorded as A_s, and Vc is used as a positive control. The hydroxyl free radical clearance rate of the sample to be tested is converted to Vc equivalent, and the hydroxyl free radical clearance rate is calculated in equation (2).

$$\text{Hydroxyl radical clearance} = [(AS - AP)/(AB - AP)] \times 100\% \quad (2)$$

2.8 Determination of Gastrointestinal Environmental Tolerance

Prepare the required plate one day in advance, take 1mL of the 48h bacterial solution, inoculate it into 9 mL of artificial stomach acid and artificial intestinal fluid respectively, shake and mix well, and then apply the plate coating. Then, culture MRS Broth with different pH at 37°C for 4h and repeat the above steps. After 48h, check the colony growth and calculate the survival rate.

$$\text{Survival rate} = \frac{\text{Number of treated live colonies}}{\text{Number of untreated live colonies}} \times 100\%$$

2.9 Bacterial Characterization

2.9.1 Self-aggregation experiment

Centrifuge 2mL of bacteria grown overnight at 10000rpm for 10min at a low temperature of 4°C. After discarding the supernatant, prepare a cell suspension in 4 mL of phosphate buffer with pH=7.0. After shaking for 1min, measure the absorbance at 600nm and incubate at 37°C for 4h. Take the upper layer and read the absorbance value at 600nm.

$$\text{Self-aggregation percentage} = \frac{\text{Absorbance value after 4 h}}{\text{initial absorbance value}} \times 100\%$$

2.9.2 Coaggregation experiment

Culture *Lactobacillus* and *Escherichia coli* in MRS Broth medium and LB medium overnight, respectively. Centrifuge 2mL of the bacterial solution at 10000rpm for 10min at 4°C. After discarding the supernatant, prepare a cell suspension in 4mL of phosphate buffer at pH = 7.0. Then, measure the absorbance at 600nm by mixing 2mL of the *Lactobacillus* suspension with 2mL of the *Escherichia coli* suspension vortex. Incubate the suspension at room temperature for 4h, and after a certain period of incubation, measure the absorbance at 600nm.

$$\text{Coaggregation percentage} = \frac{\text{Absorbance value after 4h}}{\text{Initial absorbance value}} \times 100\%$$

2.10 Data Processing

The results of this experiment were analyzed by one-way ANOVA using SPSS 20.0 statistical software. The data in the table were expressed in the form of "Average \pm SD", and $P < 0.05$ was used as the difference significance standard.

3. Result and Analysis

3.1 Isolation and Screening of *Lactobacillus*

A strain of bacteria was isolated from pickled cucumber without obvious deterioration, spoilage, and bright color. The enrichment medium sample with a gradient dilution to 10^{-3} was selected to coat the MRS Broth medium for better isolation, and the single bacterial colony cultured could be evenly distributed on the plate. After selecting the single colony with the best growth for three generations of purification, a small round colony with a smooth surface could be seen on the medium, and the surrounding area was smooth and easy to pick, and a transparent circle would be observed around the colony. The hydrogen peroxide test was negative. Number it DC - 3 and preserve it.

3.2 The Action of *Lactobacillus* to Degrade Cholesterol

3.2.1 Cholesterol degradation rate of *Lactobacillus*

The test results showed that the cholesterol degradation rate of strain DC-3 was 59.76%, which was significantly higher than that of strain 1152 under the same conditions, which was 25.32% ($P < 0.05$). The specific data are shown in Table 1.

Table 1. Determination Value of Cholesterol-Degrading Ability (%)

Strain	Cholesterol degradation rate
DC-3	40.97 \pm 1.74 a
1152	25.32 \pm 0.67 b

Note: For the same column and item, different lowercase letters of data shoulder indicate significant difference ($P < 0.05$), while the same letters of data shoulder indicate no significant difference ($P > 0.05$).

3.2.2 Detection of BSH enzyme activity

The experimental results demonstrated that a precipitation ring with a radius of 10.5 mm was formed around the filter paper inoculated with strain DC - 3. This radius was slightly smaller than the 11.0 mm observed for strain 1152 under the same conditions, yet the difference was not

statistically significant ($P > 0.05$). The specific data are presented in Table 2.

Table 2. Determination of BSH Enzyme Activity

Strain	White settling circle radius (mm)
DC-3	10.5 \pm 0.5 b
1152	11.0 \pm 1.0 a

Note: For the same column and item, different lowercase letters of data shoulder indicate significant difference ($P < 0.05$), while the same letters suggest no significant difference ($P > 0.05$).

3.3 Determination of Antioxidant Activity of *Lactobacillus*

The results indicated that the DPPH free radical scavenging rate of DC - 3 after crushing was 39.62%, which was marginally higher than the 37.73% scavenging rate of the uncrushed DC - 3. However, this difference was not statistically significant ($P > 0.05$). Similarly, the hydroxyl radical scavenging rate of DC - 3 after crushing was 46.93%, slightly exceeding the 46.71% of the uncrushed sample, with no significant difference ($P > 0.05$). The specific data are shown in Table 3.

Table 3. Numerical Record of Antioxidant Activity of Strain DC-3 (%)

Group	DPPH clearance	Hydroxyl radical clearance
The broken DC-3	39.62 \pm 0.31	46.93 \pm 0.57
Unbroken DC-3	37.73 \pm 0.69	46.71 \pm 0.24

Note: For the same column and item, different lowercase letters of data shoulder indicate a significant difference ($P < 0.05$), while the same letters of data imply no significant difference ($P > 0.05$).

3.4 Determination of Viability of *Lactobacillus*

3.4.1 Determination of bile salt tolerance

The results revealed that when the bile salt concentration was 0.3 g/L, the survival rate of *Lactobacillus* DC - 3 was 73.91%, which was lower than the 74.52% survival rate at a bile salt concentration of 0.5 g/L. However, this difference was not statistically significant ($P > 0.05$). Notably, the survival rate was significantly higher than 70.60% when the bile salt concentration reached 1 g/L ($P < 0.05$). The specific data are presented in Table 4.

Table 4. Bacteria DC-3 Survival Rate

Bile salt concentration	Strain survival rate
0.3	73.91 \pm 0.24 a

0.5	74.52±0.96 a
1	70.60±0.54 b

Note: For the same item, different lowercase letters of data shoulder label indicate significant difference ($P<0.05$), while the same letters of data shoulder label indicate no significant difference ($P>0.05$).

3.4.2 Determination of gastrointestinal environmental tolerance

The experimental findings showed that after four hours of cultivation in the simulated stomach acid medium, the survival rate of strain DC - 3 was 77.41%, and in the simulated intestinal fluid medium, it was 79.22%. The specific data are provided in Table 5.

Table 5. Tolerance of Gastrointestinal Environment of Strain DC-3 (%)

Living environment	Strain survival rate
Artificial gastric juice	77.41±0.28
Artificial intestinal fluid	79.22±0.56

Note: For the same item, different lowercase letters of data shoulder label indicate significant difference ($P<0.05$), while the same letters of data shoulder label indicate no significant difference ($P>0.05$).

3.5 Determination of Bacterial Characteristics

The experimental results demonstrated that the average self - aggregation of strain DC - 3 was 64.00%, and the average coaggregation was 25.80%. The specific data are presented in Table 6.

Table 6. Cell characteristics of strain DC-3

Group	Value
Percentage of self-aggregation	64.00±2.18
Coaggregation percentage	28.50±1.27

Note: For the same item, different lowercase letters of data shoulder label indicate significant difference ($P<0.05$), while the same letters of data shoulder label indicate no significant difference ($P>0.05$).

4. Discussion

4.1 Isolation and Screening of *Lactobacillus*

Lactobacillus is a gram - positive, spore - free, inactive bacterium with specific nutritional requirements. Through a series of steps including sample collection, gradient dilution, separation, purification, Gram staining, inclined surface preservation, salt tolerance test, and acid resistance test, a strain of *L. plantarum* DC - 3

was successfully isolated from pickle. It formed small white or light-yellow colonies on the surface or inner layer of the agar medium. Although the surface of the colonies was smooth, they were relatively small and thus difficult to observe. Therefore, an initial enrichment culture and the selection of an appropriate medium during the separation process were crucial. When calcium carbonate was added to the medium, lactic acid produced by the bacteria dissolved the calcium carbonate, forming a transparent circle, which served as the basis for separation and identification.

4.2 The Action of *Lactobacillus* to Degrade Cholesterol

An elevated serum cholesterol level is a major contributor to cardiovascular diseases such as coronary heart disease and atherosclerosis [15]. The ability to lower cholesterol is one of the important characteristics of probiotics. The cholesterol - lowering ability of *Lactobacillus* DC - 3 isolated from commercially available pickled cucumber differed significantly from that of other strains of *Lactobacillus* isolated under the same conditions. Additionally, the cholesterol removal rates of the 9 strains of *Lactobacillus* screened by David Chen et al. also varied. Bile salt hydrolase (BSH) is an enzyme with the ability to reduce cholesterol. In the test to determine the activity of BSH enzyme, the presence of obvious white precipitates around the filter paper indicated that this strain possessed BSH activity. The cholesterol clearance rate of strain DC - 3 was significantly higher than that of strain 1152, suggesting that DC - 3 had a good cholesterol - lowering ability.

4.3 Antioxidant Activity of *Lactobacillus*

When the content of free radicals in the human body becomes excessively high, it can cause damage and destruction to nucleic acids, proteins, sugars, and lipids in human cells, leading to the occurrence of certain diseases [16]. In recent years, there has been an increasing number of reports on the antioxidant activity of lactic acid bacteria. The main reason for their antioxidant activity lies in the fact that lactic acid bacteria contain superoxide dismutase and catalase, which can bind with free radicals and convert them into non - oxidizing substances. The results showed that the DPPH free radical scavenging rate of DC - 3 after crushing was 39.62%, which was slightly higher than the

37.73% scavenging rate of the uncrushed DC - 3. The scavenging rate of hydroxyl free radical was 46.93% after the strain DC - 3 was broken, which was marginally higher than the 46.71% before the strain was broken. Based on the above experimental results, the effect of fragmentation on *Lactobacillus* DC - 3 was not significant. The scavenging ability of hydroxyl radical of strain DC - 3 was much higher than that of the 35 strains of lactic acid bacteria studied by Wang Xi et al., indicating that strain DC - 3 had a certain antioxidant capacity.

4.4 The Viability of *Lactobacillus*

The salt content in the human small intestine ranges from 0.03% to 0.3%. For probiotics to adhere to the intestine and exert their probiotic properties, they must be able to withstand the high salt environment of the intestine. As shown in Table 4, when the bile salt concentration was as high as 1%, it had a significant impact on the survival of *Lactobacillus* DC - 3. In contrast, when the bile salt concentration was lower than 0.5%, it had little effect on the survival of strain DC - 3. The tolerance rate of *Lactobacillus* isolated from kimchi was higher than that reported by Huang Yanyan et al. (35.48%). These results suggest that DC - 3 has a very good bile salt tolerance. The acid resistance of probiotics used in food is also of great importance, as only probiotics that can survive in the gastrointestinal tract can play a role in promoting health. As can be seen from Table 3, the strain was able to grow in the acidic environment of the gastrointestinal tract, with a survival rate exceeding 70%. The survival rates of the strains isolated from Dongbei Sauerkraut varied widely under pH 3 conditions, ranging from 25.3% to 99.1%, indicating that DC - 3 had a good tolerance to acid.

4.5 Bacterial Properties of *Lactobacillus*

Self – aggregation [17] refers to the ability of cells to self - assemble, which plays a crucial role in cell survival. The co-aggregation [17] ability implies that the strain can attach to and absorb some harmful bacteria, such as *Escherichia coli*, thereby occupying the ecological niche of these harmful bacteria and reducing their toxic effects. Among the 10 strains of *Lactobacillus* screened from animal intestines by Song Yuxin et al., 6 strains had a self-aggregation capacity exceeding 20%. Notably, Lac.97 had a self - aggregation capacity

of 67.73%, and the co - aggregation capacity of all 10 strains was above 80%. The self - aggregation capacity of strain DC - 3 was 64.00%, slightly lower than that of Lac.97 (67.73%), and the average self - aggregation capacity was 25.80%. These results indicate that strain DC - 3 had a good self - aggregation ability, suggesting that it had a strong survival ability in harsh environments.

5. Conclusion

In this experiment, *Lactobacillus* DC - 3 isolated from preserved cucumber was investigated. The results demonstrated that DC - 3 was an excellent strain with good cholesterol - lowering ability, BSH enzyme activity, and strong antioxidant activity. It also exhibited a strong survival ability in harsh environments such as those with high bile salt concentrations and acidic conditions. Therefore, this strain could survive well in the human gastrointestinal tract. Fully exploiting its probiotic effect holds high research value in improving the intestinal environmental function, promoting the absorption of nutrients, inhibiting the absorption of cholesterol, and lowering blood lipid and blood pressure.

References

- [1] Lian, Y., J. Song, W. Mumby, et al., The Correlation between Flavor Formation and Microbial Community Dynamics during the Fermentation of Zha Cai. *Journal of the Science of Food and Agriculture*, 2024. 104(10): p. 6233-6241.
- [2] Liang, H., H. Chen, C. Ji, et al., Dynamic and Functional Characteristics of Predominant Species in Industrial Paocai as Revealed by Combined DGGE and Metagenomic Sequencing. *Frontiers in Microbiology*, 2018. 9.
- [3] Letizia, F., G. Albanese, B. Testa, et al., In Vitro Assessment of Bio-Functional Properties from *Lactiplantibacillus plantarum* Strains. *Current Issues in Molecular Biology*, 2022. 44(5): p. 2321-2334.
- [4] Wang, X., N. Zhang, D. Li, et al., Mechanism of Gastrointestinal Adaptability and Antioxidant Function of Infant-derived *Lactobacillus plantarum* BF_15 through Genomics. *Food Science and Biotechnology*, 2022. 31(11): p. 1451-1462.
- [5] De Chiara, I., R. Marasco, M. Della Gala, et

- al., Probiotic Properties of *Lactococcus lactis* Strains Isolated from Natural Whey Starter Cultures. *Foods*, 2024. 13(6): p. 957.
- [6] Wang, J., H. Ji, S. Wang, et al., Probiotic *Lactobacillus plantarum* Promotes Intestinal Barrier Function by Strengthening the Epithelium and Modulating Gut Microbiota. *Frontiers in Microbiology*, 2018. p. 9.
- [7] Sun, N., X. Ni, H. Wang, et al., Probiotic *Lactobacillus johnsonii* BS15 Prevents Memory Dysfunction Induced by Chronic High-Fluorine Intake through Modulating Intestinal Environment and Improving Gut Development. *Probiotics and Antimicrobial Proteins*, 2020. 12(4): p. 1420-1438.
- [8] Bambace, M.F., M.V. Alvarez, and M.R. Moreira, Ready-to-eat Blueberries as Fruit-based Alternative to Deliver Probiotic Microorganisms and Prebiotic Compounds. *LWT*, 2021. 142: p. 111009.
- [9] Li, X.-D., Y. Lu, C.-Y. Luo, et al., *Lactobacillus Chiyaiensis* Mediate Intestinal Microbiome and Microbiota-derived Metabolites Regulating the Growth and Immunity of chicks. *Veterinary Microbiology*, 2024. 290: p. 109969.
- [10] Wang, G., W. Huang, Y. Xia, et al., Cholesterol-lowering Potentials of *Lactobacillus* Strain Overexpression of Bile Salt Hydrolase on High Cholesterol Diet-induced Hypercholesterolemic Mice. *Food & Function*, 2019. 10(3): p. 1684-1695.
- [11] Yao, C., W. Tian, J. Song, et al., Antihyperlipidaemic Effect of Microencapsulated *Lactobacillus plantarum* LIP-1 on Hyperlipidaemic rats. *Journal of the Science of Food and Agriculture*, 2020. 100(5): p. 2007-2017.
- [12] Yuan, T., J. Wang, L. Chen, et al., Glycyrrhizic Acid Improving the Liver Protective Effect by Restoring the Composition of *Lactobacillus*. *Journal of Functional Foods*, 2019. 52: p. 219-227.
- [13] Guan, Q., T. Xiong, and M. Xie, Influence of Probiotic Fermented Fruit and Vegetables on Human Health and the Related Industrial Development Trend. *Engineering*, 2021. 7(2): p. 212-218.
- [14] Yadav, R., A.K. Puniya, and P. Shukla, Probiotic Properties of *Lactobacillus plantarum* RYPR1 from an Indigenous Fermented Beverage Raabadi. *Frontiers in Microbiology*, 2016. 7.
- [15] Proctor, S.D., M. Wang, D.F. Vine, et al., Predictive Utility of Remnant Cholesterol in Atherosclerotic Cardiovascular Disease. *Current Opinion in Cardiology*, 2024. 39(4): p. 300-307.
- [16] Katerji, M., M. Filippova, and P. Duerksen-Hughes, Approaches and Methods to Measure Oxidative Stress in Clinical Samples: Research Applications in the Cancer Field. *Oxidative Medicine and Cellular Longevity*, 2019. 2019: p. 1279250.
- [17] Kumar, R., P. Bansal, J. Singh, et al., Aggregation, Adhesion and Efficacy Studies of Probiotic Candidate *Pediococcus acidilactici* NCDC 252: A Strain of Dairy Origin. *World Journal of Microbiology and Biotechnology*, 2019. 36(1): p. 10.