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Isolation and Identification of *Lactobacillus Plantarum* DC-5 from Pickled Mustard and Its Beneficial Effects

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Abstract: The aim of this experiment was to isolate and screen a plant Lactobacillus, named DC-5, from commercially available pickled mustard and study its beneficial effects. In this experiment, selected strains were screened by the dilute coated plate method and their bile salt tolerance, gastrointestinal tolerance, cholesterol-lowering ability, antioxidant activity, antibacterial activity and bacterial properties were determined. The results showed that the survival rate of the strain at the bile salt concentration of 0.3 g/L was 70.00%, the survival rate of simulated stomach acid and intestinal fluid was about 80%; cholesterol-lowering effect was 47.03%; antioxidant activity was 50%; good aggregation ability; and can inhibit the growth of five pathogenic bacteria: Escherichia coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhi and Shigella falciparum. Studies have shown that Lactobacillus DC-5 has good beneficial effects and provides a reference value for future production of probiotic-related fermented foods.

Keywords: Brassica; *Lactobacillus Plantarum*; Beneficial Effects

1. Introduction

1.1 Introduction to Pickled Mustard

Pickled mustard is a traditional fermented food with a long history [1], serving not only as a culinary ingredient but also playing a crucial role in medicine and other fields. It possesses a unique flavor and tangy taste, along with abundant nutritional and health benefits. The production process of pickling involves special treatment, known as three pickled three pressed, which does not involve sterilization, thus resulting in the presence of rich bacteria that can be utilized as raw material for extracting *Lactobacillus plantarum*, *Bacillus amylolitica, Saccharomyces cerevisiae* and *Pediococcus* have been isolated from traditional fermented products of pickled mustard, laying the groundwork for strain isolation from pickled mustard.

1.2 Overview of *Lactobacillus Plantarum*

L. plantarum is gram-positive and spore free, and its main fermentation product is lactic acid. The optimum temperature for the growth of *L. plantarum* is 30-35℃, and the optimum pH is about 6.5. As the name suggests, its shape is curved or straight rod, single, sometimes in pairs and chain structure, belonging to the homofermentative lactic acid bacteria. Human life is inseparable from *L. plantarum* [2]. Many foods that people eat, such as vegetables, meat and vegetable cream, are made by fermentation of *L. plantarum*. After eating, people pass through the stomach and play a probiotic role in the gut. It can affect the survival of intestinal microbes and affect intestinal homeostasis [3], not only in the field of food and industrial fermentation, but also in health care has a wide range of applications.

1.3 Physiological function of *L. Plantarum*

1.3.1 Immunomodulatory effects of *L. plantarum*

The role of *L. plantarum* in immune regulation has attracted much attention. It has been reported that the strain isolated from fermented food has a strong induction effect, and the strain has been shown to be able to induce an immune response [4]. The immunomodulatory effects of L. plantarum are related to the bacteria itself and its metabolites, and sometimes even after

the death of the bacteria, it still has immunomodulatory effects. At present, there are many types of *Lactobacillus* that can cause immune regulation, such as *Lactobacillus* acidophilus, *Lactobacillus* casei, Streptococcus thermophilus and so on. Therefore, if the immunomodulatory function of *Lactobacillus* probiotics is investigated deeply, the immune mechanism of *Lactobacillus* probiotics is clarified and the evaluation of intestinal microecological regulatory function is provided with broad research space.

1.3.2 Biological barrier role of *L. plantarum*

L. plantarum not only has an inhibitory effect on gram-positive pathogens, but also can exert its inhibitory effect on negative pathogens. It can compete with pathogenic bacteria for limiting nutrients to inhibit the growth of pathogenic bacteria and regulate the composition of intestinal microecology, constituting a biological barrier. It can regulate the relationship of flora with substances such as lactic acid, bactericin and diacetyl and other metabolites on other bacteria, and maintain and ensure that the flora is in a steady state. By antagonizing the growth of pathogenic bacteria and the adhesion of pathogenic toxins, *L. plantarum* prevents their invasion and colonization [5].

1.3.3 Cholesterol-lowering effect

L. plantarum LPl003 was isolated from kimchi and the degradation rate of cholesterol in liquid medium reached 42.5%. When fermented milk made of *L. plantarum* LPl003 was fed to high-fat model mice, the serum cholesterol content of mice in the experimental group was significantly reduced, and the risk index of hyperlipidemia of mice was significantly reduced. Wang et al. [6] fed L. plantarum MA2 isolated from Kefir in Tibet to rats with high cholesterol, resulting in decreased serum total cholesterol content in rats and increased the number of *Lactobacillus* and bifidobacterium in stool of rats. The study found that patients with hypercholesterolemia could significantly reduce their cholesterol levels after taking *Lactobacillus* L. plantarum 299v. High cholesterol has always been one of the main pathogenic factors of cardiovascular diseases, so reducing serum cholesterol has become a research hotspot. In recent years, there is potential value in studying the cholesterol-lowering effect of lactic acid bacteria.

1.4 The Purpose and Significance of This Experiment

L. plantarum is closely related to human life. It is a kind of *Lactobacillus* commonly found in fermented products. It can play a beneficial role in the intestine through the stomach. *L. plantarum* is not only used in the field of food and industrial fermentation, but also has a wide range of applications in healthcare. Because pathogenic bacteria restrict the presence of nutrients, *L. plantarum* competes with pathogenic bacteria, thus limiting the growth of pathogenic bacteria and regulating the composition of intestinal microorganisms to form a biological barrier. At the same time, through the effects of substances such as organic acids, bactericins, hydrogen peroxide, and other metabolites on other bacteria, the relationship between the flora is regulated, the flora is maintained and ensured in a stable state, the immunity of the body is improved, the cholesterol content is decreased, the lactose intolerance is alleviated, and the formation of tumor cells is inhibited. With the increasing demand for people's living standards nowadays, the function of *L. plantarum* is also of great research significance. In this experiment, *L. plantarum* was screened from pickled mustard and its probiotic effect was determined, which could lay a foundation for screening *Lactobacillus* from pickled mustard and putting it into formal use by medical students and developing corresponding drugs.

2. Materials and Methods

2.1 Experimental Material

2.1.1 Experimental reagent

MRS Broth medium, AGAR, calcium carbonate, cholesterol, Twain, taurine salt, phthalaldehyde, ice acetic acid, sodium taurodeoxycholate, calcium chloride, pepsin, sodium chloride, trypsin, sodium bicarbonate, sodium hydroxide, anhydrous ethanol, potassium hydroxide, n-hexane, concentrated sulfuric acid, (all reagents are analytically pure), distilled water is used to prepare the reagents.

2.1.2 Main experimental instrument

In addition to some precision instruments, we also use petri dishes, inoculation rings, conical bottles, beakers, volumetric bottles, beakers, glass rods, measuring cylinders, plastic eyedroppers and other conventional laboratory

glass equipment; Disposable gloves, disposable pipette head and other consumables. (The instruments used are shown in Table 1)

Table 1. Main Experimental Instrument

Experimental instrument	Machine type	Manufacturer
Electronic balance	HZF-A1000	Fuzhou Huazhi Scientific Instrument Co., LTD
Analytical balance	AR224CN	Ohaus Instrument (Changzhou) Co., LTD
Refrigerator	BCD-137TMPF	Qingdao Haier Co., LTD
Pipette gun	Research plus	Eppendorf GMBH
Autoclave	YXQ-LS-30SSII	Shanghai Boxun Industrial Co., LTD. Medical equipment factory
Thermostatic water bath	KW-1000DC	Jintan city Zhongda instrument factory
Ultra-clean table	BSC-1000IIA2	Sujing Group Suzhou Antai Air Technology Co., LTD
Biochemical incubator	SPX-100B-Z	Shanghai Boxun Industrial Co., LTD. Medical equipment factory
Ultraviolet visible spectrophotometer	WFZ—UV-2000	Uniko (Shanghai) Instrument Co., LTD

2.1.3 Experimental related solutions and their formulations (Table 2)

Table 2. Related Solution Formulation

Solution	Formula
MRS Broth	52.24g/L MRS broth is heated with medium	1.5% Agar until completely dissolved, then 1%$CaCO_3$ is added.
Cholesterol-MRS Medium	1g/L cholesterol and 2% Tsum were completely dissolved by heating, 3g/L bile salt was added, and the prepared cholesterol-Tsum solution and MRS Broth medium were stored to 1L for use
Bile salt MRS Medium	0.5% sodium taurine deoxycholate and 0.37g/L$CaCl_2$ were mixed into MRS Broth medium
Phthalaldehyde solution	Weigh 0.05g of phthalaldehyde and volume it with glacial acetic acid to 100ml. This solution should be used and stored away from light
DPPH reserve liquid	Weigh 20mgDPPH standard and fill it with anhydrous ethanol into a 10mL brown volumetric bottle and store at -20℃
DPPH working fluid	Take 1mL DPPH reserve liquid and place it in a 100ml brown volumetric bottle with a concentration of 0.02mmol/L
Artificial stomach acid formula	Pepsin (1:500) 3.5g/L, NaCL2g/L, adjusted with hydrochloric acid to pH=3
Artificial intestinal fluid	Trypsin (1:250) 1g/L, bile salt 1.2g/L, Nacl 4.4g/L, $NaHCO_3$ 2.2g/L, adjusted with NaOH to pH=8. Gastrointestinal environment: Mix of the above two

2.2 Experimental Method

2.2.1 Strain isolation and screening

The samples of 10g pickled mustard were put into a sterile mortar for grinding, the ground crushed pickled mustard and the juice were placed in 50mL sterile water, and then the mixed solution was diluted in gradient, and the samples with different dilution ratios were inoculated into MRS Broth medium, and incubated at 37℃ for 48h. A single colony with a large transparent circle was selected from the cultured medium for plate stripe culture, and the strains obtained after 3 generations of culture (purification) were screened by touch enzyme test. A part of the colony was selected on a slide with a sterilized bamboo skewer in a sterile environment, and a drop of 30%H_2O_2 was absorbed on the selected bacteria. If there was bubbling, it was positive, while no bubbling was

negative. The strain with negative contact enzyme test results was named DC-5, and then inoculated in MRS Broth medium, incubated at 37℃ for 48h, and stored on the inclined side of a test tube at 4℃ for reserve.

2.2.2 Determination of bile salt tolerance

A plate was prepared one day in advance, and bovine bile salts with concentrations of 0.3g/L, 0.5g/L and 1.0g/L were added to the prepared liquid MRS Broth medium, which were sterilized at 120℃ for 15min and then used. Take 1mL of the bacterial solution cultured for 48h and place it in MRS Broth medium containing 9mL of bile salts of different concentrations. After mixing it evenly, dilute it ten times. After mixing it evenly again, apply it for counting. Calculate survival rate (survival rate = number of colonies after 4h/number of colonies at inoculation ×100%)

2.2.3 Determination of gastrointestinal tolerance

Prepare the required plate one day in advance to prepare the artificial gastric acid solution and the artificial intestinal fluid solution. The two solutions were mixed in a simulated gastrointestinal environment. The 1mL culture 48h bacterial solution was inoculated into 9mL of the above liquid, and the sample was coated at inoculation and 4h after inoculation respectively, and the survival rate was calculated (survival rate = number of treated live colonies/number of untreated live colonies ×100%). This experiment is based on the experimental methods of Peng Can and Zhou Haizhu et al., and slightly modified. Prepare the required plate one day in advance, take 1mL of the 48h bacterial solution, inoculate it into 9mL of artificial stomach acid and artificial intestinal fluid respectively, shake and mix well, and then apply the plate coating. Then MRS Broth with different pH was cultured at 37℃ for 4h, and the above steps were repeated three times. After 48h, colony growth was checked and survival rate was calculated (survival rate = number of colonies after 4h/number of colonies at inoculation ×100%).

2.2.4 Determination of cholesterol-lowering ability

L. plantarum was inoculated into 19mL of cholesterol-MRS liquid broth broth with 1mL culture solution for 48h. After culture at 37℃ for 24h, the cholesterol content of *L. plantarum* was measured by phthalaldehyde method. Add 1mL sample, 3mL anhydrous ethanol and 2mL 50%KOH into the test tube respectively, and place them in a water bath at 60℃ for 30min. After cooling to room temperature, add 3mL normal hexane for full shock, then add 3mL distilled water for fine stratification, absorb the upper liquid and dry at 60℃ to extract cholesterol. 2mLOPA reagent was added to the dried sample, and after reaction at room temperature for 10min, 2mL concentrated sulfuric acid was added, and after shock for 20s, the reaction at room temperature for 10min. The absorbance value at 550nm was measured, the cholesterol content was determined, and the degradation rate of cholesterol was calculated. Repeat the preceding steps three times. Cholesterol degradation rate is calculated as follows:

Cholesterol degradation rate= (Cholesterol degradation/ Initial cholesterol) ×100%

2.2.5 Determination of antioxidant activity

DPPH free radical and hydroxyl free radical scavenging capacity of *L. plantarum*, i.e. antioxidant activity [7]. Take a 0.2mL sample, add 2mL (0.02mmol/L) DPPH working liquid, and after the reaction at 6000rpm for 30min, centrifuge for 10min, measure the absorbance at 517 nm wavelength and record it as A0. Replace the sample with anhydrous ethanol and record it as A1. Replace DPPH with anhydrous ethanol and record it as Ai. Use Vc to make the standard curve, with Vc as the equivalent. The experiment was repeated three times. The DPPH free radical clearance rate is calculated in equation (1):

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

1 mL 2.5 mmol/L of phenphenoline, 1 mL 0.02 mmol/L of PBS phosphate buffer and 1 ml of distilled water were placed in a test tube, mixed well and then added 1 mL 2.5 mmol/L of Fe_2SO_4. After sufficient reaction, 1 mL 20 mmol/L of hydrogen peroxide was added. After reaction at 37℃ for 1.5h, the absorbance measured at 536nm was recorded AS AP, the H_2O_2 solution was replaced by distilled water as AB, and the solution to be measured was replaced by distilled water as as as. Vc was used as the positive control standard curve, and the clearance rate of hydroxyl radical of the sample to be measured was converted to Vc equivalent. The experiment was repeated three times. Hydroxyl radical scavenging rate is calculated in equation (2).

Hydroxyl radical clearance $= [(A_s-A_p)/(A_B-A_P)] \times 100\%$

2.2.6 Determination of bacteriostatic activity

Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella flexneri and Bacillus cereus were used as indicator bacteria for laboratory culture. The activated lactic acid bacteria were inoculated into the sterilized MRS Broth liquid medium at 3% inoculation rate, cultured at 37℃ for 48h, centrifuged at 4℃ and 5000r/min for 10min, and the fermentation supernatant was taken and stored at 4℃ for later use. Take 3 to 4 sterilized Oxford cups and place them evenly on the petri dish. After the indicator bacteria solution activated 2 to 3 times, pour them into solid culture at about 40℃, shake them well and pour them onto the plate. After solidification, culture them at 37℃ for 24h for reserve use. The solidified indicator bacteria plate was placed on a horizontal table, the Oxford cup was taken out and inserted into the medium, and the same amount of lactic acid bacteria fermentation supernatant was added in drops respectively. After culture at 37℃ for 48h, the diameter of the antibacterial circle was measured, and 75% ethanol solution and MRS Broth liquid medium were used as the control [8]. Repeat the test three times.

2.2.7 Bacterial characteristics

The absorbance value of *L. plantarum* DC-5 at 600nm was determined to determine the self-aggregation and co-aggregation performance of the strain [8-10]. Each experiment was repeated three times according to the same criteria.

Self-aggregation percentage = (absorbance value after 4h/initial absorbance value) ×100%

Coaggregation percentage = (absorbance value after 4h/initial absorbance value) ×100%

2.2.8 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±SD", and $P<0.05$ was used as the difference significance standard.

3 Results and Analysis

3.1 Isolation and Screening of *L. Plantarum*

A *L. plantarum* was isolated from fresh pickled mustard without obvious deterioration, putridity and bright color, and the *Lactobacillus* was coated in MRS Broth medium suitable for growth, and the sample with the best separation effect was selected from its enriched medium. In this experiment, the concentration gradient of 10^{-4} had the best separation effect, which could not only culture a single colony, but also distribute uniformly in the plate. The single colony with the best growth was selected and purified for three generations. The morphology of the colony growing in the medium was white and round. The surface is wet and smooth; A single colony is easy to pick and has tidy edges. A transparent ring is produced on the separating plate. Number it DC-5 and preserve it.

3.2 Determination of Probiotic Effect of *L. Plantarum*

3.2.1 Determination of bile salt tolerance

The results showed that the survival rate of DC-5 at 0.3g/L was 70.00%, which was significantly higher than 58.12% at 0.5g/L ($P<0.05$), and was also significantly higher than 43.15% at 1.0g/L ($P<0.05$). The survival rate of DC-5 at 0.5g/L was 58.12%, which was significantly higher than that of 43.15% at 1.0g/L ($P<0.05$). Specific data are shown in Table 3.

Table 3. DC-5 Strain Viability

Bile salt concentration (g/L)	Survival rate of DC-5
0.3	70.00±1.20[a]
0.5	58.12±0.88[b]
1	43.15±1.33[c]

Note: For different concentrations in the same column, different lowercase letters of shoulder label indicate significant difference ($P<0.05$), while no shoulder label or the same letter of shoulder label indicate no significant difference ($P>0.05$).

3.2.2 Determination of gastrointestinal tolerance

The results showed that the survival rate of DC-5 in artificial gastric fluid was 81.10%, which was slightly lower than that in artificial intestinal fluid (81.60%), and the difference was not significant ($P>0.05$). Specific data are shown in Table 4.

Table 4. Tolerance of Gastrointestinal Environment of Strain DC-5

Gastrointestinal environment	Survival rate of DC-5
Artificial gastric juice	81.10±1.51
Artificial intestinal fluid	81.60±1.06

Note: In the same column and different environments, different lowercase letters of shoulder label indicate significant difference ($P<0.05$), while no shoulder label or the same letter of shoulder label indicate no significant

difference (P>0.05).

3.2.3 Determination of cholesterol-lowering ability

The results showed that the cholesterol degradation rate of DC-5 was 47.03%, which was significantly higher than 20.62% in the control group (acetic acid + sulfuric acid), and the difference was significant (P<0.05). Specific data are shown in Table 5.

Table 5. Determination of Cholesterol Degradation Ability

Group	Cholesterol degradation rate
Control	20.62±0.76[a]
DC-5	47.03±0.67[b]

Note: For different items in the same column, different lowercase letters of shoulder label indicate significant difference (P<0.05), while no shoulder label or the same letter of shoulder label indicate no significant difference (P>0.05).

3.2.4 Determination of antioxidant activity

The results showed that the DPPH free radical scavenging rate of DC-5 after crushing was 55.25%, which was significantly higher than that of DC-5 without crushing (P<0.05). The hydroxyl radical scavenging rate of DC-5 after crushing was 53.76%, which was significantly higher than that of DC-5 without crushing, which was 38.38%, and the difference was significant (P<0.05). Specific data are shown in Table 6.

Table 6. Free Radical Scavenging Rate of Strain DC-5

Group	DPPH scavenging rate
The broken DC-5	55.25±1.61[a]
Unbroken DC-5	43.92±1.06[b]

Note: For different items in the same column, different lowercase letters of shoulder label indicate significant difference (P<0.05), while no shoulder label or the same letter of shoulder label indicate no significant difference (P>0.05).

3.2.5 Determination of bacteriostatic activity

The experiment showed that DC-5 had inhibition zone on Escherichia coli, Staphylococcus aureus, Salmonella typhus, Shigella flexneri and Bacillus cereus. The inhibition zone diameter of DC-5 on Bacillus cereus was 14.8mm, slightly lower than that of Bacillus cereus (15.6mm), and the difference was not significant (P>0.05). The diameter of inhibition zone was 15.2mm higher than that of Shigella flexneri, and the difference was not significant (P>0.05). The inhibitory zone diameter of DC-5 against Salmonella typhi was 10.1mm, which was significantly lower than that of Bacillus cereus (14.8mm), that of Escherichia coli (15.6mm) and that of Shigella fowleri (15.2mm), and the differences were significant (P<0.05). It was slightly lower than that of Staphylococcus aureus (11.1mm), the difference was not significant (P>0.05). The inhibitory zone diameter of DC-5 against Staphylococcus aureus was 11.0mm, which was significantly lower than that of Bacillus cereus (14.8mm), that of Escherichia coli (15.6mm) and that of Shigella fowleri (15.2mm), and the differences were significant (P<0.05). DC-5 was more effective against Bacillus cereus, Escherichia coli and Shigella flexneri than Staphylococcus aureus and Salmonella typhi. Specific data are shown in Table 7.

Table 7. Inhibitory Effect of Strain DC-5 on Different Indicator Bacteria (mm)

Strain	Inhibition zone diameter
Bacillus cereus	14.8±0.8[a]
E. coli	15.6±1.06[a]
S. aureus	11.0±0.8[b]
s. typhi	10.1±0.1[b]
Sh. flexneri	15.2±0.3[a]

Note: For different strains in the same industry, different lowercase letters of shoulder label indicated significant difference (P<0.05), while no shoulder label or the same letter of shoulder label indicated no significant difference (P>0.05).

3.2.6 Bacterial characteristics

The results showed that the percentage of self-aggregation and co-aggregation of strain DC-5 was 52.76% (P<0.05) and 51.89% (P<0.05), respectively, with no significant difference (P<0.05). Specific data are shown in Table 8.

Table 8. Self-and Co-Aggregated Percentages

Group	Self-aggregation percentage	Total aggregation percentage
DC-5	61.06±1.89	60.63±1.72

Note: For different items in the same industry, different lowercase letters of shoulder label indicate significant difference (P<0.05), while no shoulder label or the same letter of shoulder label indicate no significant difference (P>0.05).

4. Discussion

4.1 Isolation and Screening of *L. Plantarum* DC-5

A *L. plantarum* was isolated from fresh pickled

mustard without obvious deterioration, spoilage and bright color, and it was named DC-5. Because the *Lactobacillus* isolated from pickled mustard in this experiment could not completely guarantee that the strain was not mixed with miscellaneous bacteria, gradient dilution culture was needed. *L. plantarum* was coated in MRS Broth medium suitable for growth, and the sample with the best separation effect was selected from its enriched medium. In this experiment, the concentration gradient of 10^{-4} was the best separation effect. Under the concentration gradient of 10^{-4}, not only a single colony could be cultivated, but also could be evenly distributed in the plate. The morphology of the selected colonies in the medium was white and round. The surface is moist and smooth; A single colony is easy to pick and has neat edges; The strains produced by transparent circles on the separation plate were cultured. The purified *Lactobacillus* cultured for three generations was tested by catalase, and the non-bubbling strain DC-5 was obtained and stored in a suitable preservation method for later use.

4.2 Analysis of Probiotic Effect of *L. Plantarum* DC-5

4.2.1 Bile salt tolerance analysis

The bile salt tolerance of the strains in the simulated bile environment showed a tendency to increase [11]. The normal bile salt concentration in human body is 0.3%-0.5%g/L, while the survival rate of strain DC-5 decreases at 0.3g/L, 0.5g/L and 1.0g/L respectively, and the survival rate is the highest at 0.3g/L. Therefore, *Lactobacillus* has good bile salt tolerance within a certain bile salt concentration range [12]. The experiment of Yu Huiming et al. showed that *Lactobacillus* also had a strong tolerance to bile salt. Although bile salt had an inhibitory effect on the growth of *Lactobacillus*, *Lactobacillus* could survive in MRS Culture solution with a bile salt concentration of 1.0%, but the survival rate was lower than that under other concentrations.

4.2.2 Gastrointestinal tolerance analysis

The pH of human gastric juice ranges from 1.5 to 4.0, and its pH changes depending on what is ingested, sometimes even greatly fluctuating, and usually only acidophilic microorganisms can grow in this low acid environment. *Lactobacillus* must be able to accept changes in the pH of gastric fluid at any time in order for it to function in the human gut. Yu Huiming et al. showed that the survival rate of *Lactobacillus* before screening was not higher than 2% after being cultured in MRS Solution with pH2.5 and pH3.0 for 3h, but the survival rate of *Lactobacillus* after acid resistant screening was 30% and more than 80% respectively under the same conditions, indicating that the experimental *Lactobacillus* had strong resistance under acidic conditions. There must be gastric acid and bile salt environment in the digestive tract of animals. If *Lactobacillus* wants to survive in the gut and play a probiotic effect, it is a prerequisite to resist strong acid and high concentration of bile salt environment. The survival rate of the strain DC-5 selected in this experiment was as high as 80% in the artificial gastric juice and intestinal juice, which could ensure that *Lactobacillus* could pass through the stomach smoothly and play a role in the intestine. Moreover, the strain selected in this experiment had a strong inhibitory effect on pathogenic bacteria, especially gram-negative bacteria, and was safe and non-toxic to animals.

4.2.3 Analysis of cholesterol-lowering ability

Nowadays, people have higher and higher requirements for quality of life, and long-term intake of foods with high cholesterol content often causes a series of diseases, so people begin to choose low-calorie and low-protein foods and enter the health mode. The use of microorganisms to produce low-cholesterol food and the use of microorganisms to degrade cholesterol in food have gradually emerged [13], and the cholesterol-lowering ability of *L. plantarum* has extremely important research significance here. The cholesterol degradation rate of *Lactobacillus* screened from traditional pickled mustard reached 50% in vitro, while that of the control group was less than half. Therefore, *Lactobacillus* DC-5 has good cholesterol-lowering ability. Excluding the possible influence of the components of the medium itself and some environmental factors on the experimental results, it can be considered that the mechanism of Lactobacillus degrading cholesterol in human body includes assimilation theory, precipitation theory and infiltration through cell membrane.

4.2.4 Analysis of antioxidant activity

L. plantarum can improve the flavor and nutritional value of food, so its fermented food has attracted much attention, but also has been loved by people [14] The cause of human

malignant diseases has been concerned, in fact, it is due to the excessive production of oxygen free radicals in the body but cannot be removed in time. If the content of free radicals in the human body is too high, it will destroy the substances in the cells, and the destruction of the substances will lead to a series of diseases in the human body [15]. *Lactobacillus* has formed its own antioxidant system over a long period of evolution. Many in vivo and in vitro experiments have shown that *Lactobacillus* has very obvious antioxidant activity. Because there are superoxide dismutase and catalase in *Lactobacillus*, they can combine with free radicals to transform into non-oxidizing substances, and exert their antioxidant activity to prevent some free radicals from harming the human body [16]. The clearance rate of DPPH free radical and hydroxyl free radical of *L. plantarum* NJAU-01 was 20%-30%, and the clearance rate of hydroxyl free radical of *Lactobacillus* strain L3 was as high as 80%. Even if the clearance rate was low, there was still free radical scavenging ability, which could also reduce the damage caused by free radicals. In this experiment, the free radical scavenging ability of strain DC-5 after crushing was as high as 50%, but the free radical scavenging ability of strain DC-5 after not crushing was less than 45%, because the superoxide dismutase and catalase in *L. plantarum* after crushing could better combine with free radicals and exert their antioxidant activities. Strain DC-5 had strong free radical scavenging ability in vitro.

4.2.5 Analysis of antibacterial activity

In this experiment, *L. plantarum* DC-5 had inhibitory effect on all the 5 indicator bacteria in the experiment. Some researchers have shown that *L. plantarum* BM-1 has different degrees of inhibition on 12 species of Escherichia coli. Although the antibacterial spectrum of *L. plantarum* is relatively broad, there are still obvious differences in the antibacterial effect. The antibacterial effect of *L. plantarum* DC-5 on Staphylococcus aureus and Salmonella typhi was lower than that of the other three bacteria. *L. plantarum* can produce organic acids, native lactic acid, bacteriocin and other substances that have killing effects on other pathogens, and then exert its antibacterial effect [17]. The antibacterial effect of strain DC-5 in this experiment needs to be further studied.

4.2.6 Bacterial characteristics

Autoaggregation, the ability of cells to self-assemble, improves cell survival. The co-aggregation ability means that the strain absorbs harmful bacteria such as E. coli and occupies the position of harmful bacteria to reduce the toxic effect of harmful bacteria. More than half of the lactic acid bacteria screened from animal intestines had the ability of self-aggregation, and some strains had the ability of self-aggregation as high as 60%, and all had the ability of co-aggregation as high as 80%. If the pathogenic bacteria in the intestine accumulate too much in the body, it will destroy the intestinal homeostasis, resulting in a series of diseases. In order to reduce the damage of pathogenic bacteria to intestinal homeostasis, the specific proteins on the surface of *Lactobacillus* can first snatch the attachment site of pathogenic bacteria, and thus co-aggregate with pathogenic bacteria. In this experiment, the self-aggregation and co-aggregation abilities of strain DC-5 reached 61.06% and 60.63%, so the strain DC-5 has strong self-aggregation and co-aggregation ability, and can play its probiotic role in human body.

4.3 Experimental Influencing Factor

In the process of studying the isolation, identification and probiotic effect of *L. plantarum* DC-5 in pickled mustard, the inoculation amount and survival rate of the bacteria, as well as the test environment temperature and equipment will affect the experimental results. Uncontrollable factors such as the origin of the vegetables used in the production of pickled mustard in the market, the production date of pickled mustard, the additives used, or accidental errors in the operation due to factors such as instruments and reagents during the test process will inevitably have a certain impact on the test results.

5. Conclusion

In this study, *L. plantarum* DC-5 was isolated and screened from commercially available pickle, and its bile salt tolerance, gastrointestinal tolerance, cholesterol-lowering ability, antioxidant activity, antibacterial activity and bacterial characteristics were analyzed. The results showed that DC-5 had good bile salt tolerance, gastrointestinal tolerance, co-aggregation ability, free radical scavenging ability, self-aggregation ability and

co-aggregation ability, and could exert its antibacterial activity, and then play a good probiotic role in the human gastrointestinal tract. This experiment can provide reference value for further research and development of *L. plantarum*.

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