Lactiplantibacillus plantarum JN-7, Bacterial Powder, Preparation Method, Probiotic Composition and Use Thereof

This application claims priority to a Chinese patent application filed on June 14, 2024 with application number 202410775747.8 and the invention title "*Lactiplantibacillus plantarum* JN-7, bacterial powder, preparation method, probiotic composition and uses thereof", the contents of which should be understood to be incorporated herein by reference.

Technical Field

The present invention relates generally to the field of biological application technology, in particular to a *Lactiplantibacillus plantarum* JN-7, bacterial powder, preparation method, probiotic composition and uses thereof.

Background

Probiotics are a kind of active microorganisms that are beneficial to a host by colonizing in human body and changing the composition of flora in a certain part of the host. Probiotics promote nutrient absorption and maintain intestinal health by regulating the host mucosal and systemic immune functions or by adjusting the balance of intestinal flora, thus producing single microorganism or mixed microorganisms with clear composition that are beneficial to health.

Lactiplantibacillus plantarum is a common kind of probiotic, which is widely used in fermented dairy products, meat products, plant products and baked foods. Its main function is to produce lactate, reduce pH, prevent the reproduction of spoilage bacteria, and improve the flavor and texture of products, etc. In recent years, studies have found that *Lactiplantibacillus plantarum* has good probiotic function, which, among others, can inhibit gastrointestinal tract pathogenic bacteria including *Helicobacter pylori*, improve gastrointestinal function and metabolism, enhance intestinal barrier function, and reduce the risk of infection.

The stability and activity of probiotics in food are closely related to the health care effects to which the probiotics can achieve. There is an urgent need to develop probiotics with good

stability and long-term preservation.

Summary

The present application provides a *Lactiplantibacillus plantarum* JN-7, wherein the *Lactiplantibacillus plantarum* JN-7 has a taxonomic designation of *Lactiplantibacillus plantarum*, and a Depositary Accession Number of CGMCC No. 30605.

In another aspect, the present application also provides a bacterial powder prepared from the *Lactiplantibacillus plantarum* JN-7 described herein.

In another aspect, the present application also provides a method for preparing the bacterial powder described herein, comprising the steps of:

1) fermenting the *Lactiplantibacillus plantarum* JN-7 described herein;

2) centrifuging the fermentation product to obtain a precipitate, and freeze-drying the precipitate to obtain the bacterial powder.

In another aspect, the present application also provides a probiotic composition comprising the *Lactiplantibacillus plantarum* JN-7 described herein or the bacterial powder described herein.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN-7 described herein, the bacterial powder described herein, or the probiotic composition described herein in the preparation of an anti-inflammatory medicament.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN-7 described herein, the bacterial powder described herein, or the probiotic composition described herein in the preparation of foods or nutraceuticals.

The viable cells survived in probiotic powder are closely related to the quality and production cost of probiotic products. Strains with good stability can increase the number of viable cells in the probiotic powder, which is helpful to improve the quality of the probiotic powder and reduce the cost.

The present application provides a strain of *Lactiplantibacillus plantarum* that has good yield, stability, safety, acid, bile salt and gastrointestinal tract resistance, and antibiotic sensitivity, which is named as *Lactiplantibacillus plantarum* JN-7. It was deposited in: China General

Microbiological Culture Collection Center, address: Institute of Microbiology, Chinese Academy of Sciences, No. 3, Yard 1, Beichen West Road, Chaoyang District, Beijing, China, and the deposit date is May 13, 2024. Depositary Accession Number is CGMCC No. 30605.

Through the analysis of basic characteristics (growth curve, acid resistance, bile salt resistance, gastrointestinal fluid resistance), safety evaluation (antibiotic sensitivity, hemolytic ability, toxigenic ability, cytotoxicity), adhesion evaluation, anti-inflammatory effect evaluation, production performance evaluation (fermentation yield and freeze-drying yield) and stability test (accelerated storage stability test) of the strain, *Lactiplantibacillus plantarum* JN-7 was found to be a safe strain with excellent production performance, high stability and anti-inflammatory efficacy. The bacterial powder produced from the bacteria can maintain stability and activity for a long time.

Additional features and advantages of the present application will be set forth in the description which follows, and in part will become apparent from the description, or may be learned by practice of the application. Other advantages of the present application may be realized and obtained by solutions described in the description and the drawings.

Brief Description of Drawings

The accompanying drawings are used to provide an understanding of the technical solutions of the present application, and constitute a part of the specification. They are used to explain the technical solutions of the present application together with the embodiments of the present application, and do not constitute a limitation to the technical solutions of the present application.

FIG. 1 is a circular chart of the genome of the *Lactiplantibacillus plantarum* JN-7 in an example of the present application. The circle chart shows seven types of information from the outside to the inside: the first circle is genome position information, the second circle is GC content information, the third circle is coding genes on the positive strand (marked in red), the fourth circle is coding genes on the negative strand (marked in green), the fifth circle is ncRNA on the positive strand (marked in blue), the sixth circle is ncRNA information on the negative strand (marked in purple), and the seventh circle is long fragment repeat information on the genome (marked in orange).

FIG. 2 is a growth curve of the *Lactiplantibacillus plantarum* JN-7 from OD 0.1 at 37 °C and 200 rpm in an example of the present application.

FIG. 3 shows the hemolytic capacity results of the *Lactiplantibacillus plantarum* JN-7 in an example of the present application.

FIG. 4 shows the interaction of the *Lactiplantibacillus plantarum* JN-7 with intestinal epithelial cell HT-29 in an example of the present application. Herein, a: the *Lactiplantibacillus plantarum* JN-7 did not reduce the viability of HT-29 cells. Data represent the survival rates of cells co-incubated with the *Lactiplantibacillus plantarum* JN-7 for 18 h and cells in culture medium alone (n = 6). b: Adhesion ratio of the *Lactobacillus rhamnosus* LGG and the *Lactiplantibacillus plantarum* JN-7 to HT-29 cells. Numerical values represent the adhesion rate of LGG and the *Lactiplantibacillus plantarum* JN-7 after incubation with HT-29 for 4 h. Data were expressed as Mean ± Sem., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

FIG. 5 shows the anti-inflammatory ability of the *Lactiplantibacillus plantarum* JN-7 to modulate the production of inflammatory factors by LPS-stimulated THP-1 cells in an example of the present application. Herein, a: interleukin 6 (IL)-6 mRNA; b: IL-8 mRNA; c: IL-10 mRNA. Data were expressed as Mean ± Sem., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

FIGS. 6A-6D show the number of viable cells and water activity of the *Lactiplantibacillus plantarum* JN-7 powders (FIG. 6A), the *Lactobacillus rhamnosus* LGG (FIG. 6B), and 15 other *Lactiplantibacillus plantarum* powders (FIGS. 6C-6D) during storage at 40 °C and 75% humidity in an example of the present application.

Detailed Description

Unless otherwise indicated, technical and scientific terms used herein have the same meanings as commonly understood by those skilled in the art to which this application belongs. When an amount, concentration, or other value or parameter is expressed in the form of a range, a preferred range, or a preferred upper numerical limit and a preferred lower numerical limit, it should be understood to be equivalent to specifically disclosing any range obtained by combining any pair of upper range limit or preferred numerical values with any lower range limit or preferred numerical values, regardless of whether the range is specifically disclosed or not. Unless

otherwise specified, the numerical ranges set forth herein are intended to include the endpoints of the range and all integers and fractions (decimals) within the range.

The terms “about,” “approximately,” when used with a numerical variable, generally refer to the value of that variable and all values of that variable are within experimental error (e.g., within a 95% confidence interval of the mean) or within ±10% of the specified value, or a wider range.

The expression “comprising” or similar expressions thereof which are synonymous such as “including”, “containing”, and “having”, are open-ended and do not exclude additional unrecited elements, steps, or ingredients. The expression “consisting of” excludes any element, step, or ingredient that is not specified. The expression “consisting essentially of” means that the scope is limited to the specified elements, steps, or components, plus the optional elements, steps, or ingredients that do not materially affect the basic and novel characteristics of the claimed subject matter. It is to be understood that the expression “comprising” encompasses the expressions “consisting essentially of” and “consisting of”.

The expression “at least one” or “one or more” refers to 1, 2, 3, 4, 5, 6, 7, 8, 9 or more.

Research on improving the stability of functional strains is mostly achieved through the development of bacterial powder protectants. Due to the inherent stability of each strain, protectants have limited effects in stabilizing probiotic powders. The development of strains with good stability for use in compositions of commercial probiotic powders is another way to solve the problem of low bacterial activity of probiotic products during their shelf life.

Based on this, the present application provides a *Lactiplantibacillus plantarum* JN-7, wherein the *Lactiplantibacillus plantarum* JN-7 has a taxonomic designation of *Lactiplantibacillus plantarum*, and a Depositary Accession Number of CGMCC No. 30605.

In another aspect, the present application also provides a bacterial powder prepared from the *Lactiplantibacillus plantarum* JN-7 described herein.

In another aspect, the present application also provides a method for preparing the bacterial powder described herein, comprising the steps of:

1) fermenting the *Lactiplantibacillus plantarum* JN-7 described herein;

2) centrifuging the fermentation product to obtain a precipitate, and freeze-drying the precipitate to obtain the bacterial powder.

In some embodiments, the method for preparing the bacterial powder described herein comprises the steps of:

1) fermentation culturing the *Lactiplantibacillus plantarum* JN-7 described herein in a bioreactor, with pH 4.5-5.3, temperature 35-40 °C, stirrer speed 160-200 rpm;

2) centrifuging the fermentation product to obtain a precipitate, mixing the precipitate with a protectant, freeze-drying in a vacuum freeze dryer and pulverizing into powder to obtain bacterial powder.

In another aspect, the present application also provides a probiotic composition comprising the *Lactiplantibacillus plantarum* JN-7 described herein or the bacterial powder described herein.

In some embodiments, the probiotic composition further comprises one or more probiotics selected from: *Bifidobacterium, Lactobacillus, Lacticaseibacillus, Limosilactobacillus, Lactiplantibacillus, Ligilactobacillus, Latilactobacillus, Streptococcus, Lactococcus, Propionibacterium, Acidipropionibacterium, Weizmannia, Mammaliicoccus, Staphylococcus, Kluyveromyces, Leuconostoc, Pediococcus* and *Bacillus subtilis* DE111.

In some embodiments, the probiotic composition further comprises one or more probiotics selected from: *Bifidobacterium adolescentis, Bifidobacterium animalis* subsp. *animalis, Bifidobacterium animalis* subsp. *lactis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum* subsp. *longum, Bifidobacterium longum* subsp. *infantis, Lactobacillus acidophilus, Lactobacillus crispatus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus delbrueckii* subsp. *lactis, Lactobacillus gasseri, Lactobacillus helveticus, Lactobacillus johnsonii, Lactobacillus kefiranofaciens* subsp. *kefiranofaciens, Lacticaseibacillus casei, Lacticaseibacillus paracasei, Lacticaseibacillus rhamnosus, Limosilactobacillus fermentum, Limosilactobacillus reuteri, Lactiplantibacillus plantarum, Ligilactobacillus salivarius, Latilactobacillus curvatus, Latilactobacillus sakei, Streptococcus salivarius* subsp. *thermophilus, Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *lactis biovar diacetylactis, Lactococcus cremoris, Propionibacterium freudenreichii subsp. shermanii, Acidipropionibacterium acidipropionici, Leuconostoc mesenteroides* subsp. *mesenteroides,*

Pediococcus acidilactici, Pediococcus pentosaceus, Weizmannia coagulans, Mammaliicoccus vitulinus, Staphylococcus xylosus, Staphylococcus carnosus, Kluyveromyces marxianus, and *Bacillus subtilis* DE111.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN-7 described herein, the bacterial powder described herein, or the probiotic composition described herein in the preparation of an anti-inflammatory medicament. In some embodiments, the medicament is administered orally.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN-7 described herein, the bacterial powder described herein, or the probiotic composition described herein in the preparation of foods or nutraceuticals.

In some embodiments, the foods or nutraceuticals are dairy products, soy products, meat products, fruits and vegetable products, beverages, or snacks. In some embodiments, the foods or nutraceuticals further comprise edible adjuvants.

In some embodiments, the foods are health foods. Or the foods comprise dairy products, soy products, meat products, or fruit and vegetable products. Or the foods are beverages or snacks. In some embodiments, the foods comprise the *Lactiplantibacillus plantarum* JN-7 described herein and edible adjuvants.

The present application describes a plurality of embodiments, but the description is exemplary and not restrictive, and it will be apparent to those skilled in the art that there may be more embodiments and implementations within the scope of the embodiments described in the present application. Although many possible combinations of features are shown in the drawings and discussed in the Detailed Description, many other combinations of the disclosed features are also possible. Unless specifically limited, any feature of any embodiment may be used in combination with any other feature in any other embodiment, or may replace any other feature in any other embodiment.

The present application includes and contemplates combinations with features known to those of ordinary skill in the art. The embodiments and features that have been disclosed in this application may also be combined with any conventional features to form a unique inventive solution as defined by the claims. Any feature of any embodiment may also be combined with

features from other inventive solutions to form another unique inventive solutions defined by the claims. Accordingly, it should be understood that any of the features shown and/or discussed in this application may be implemented alone or in any suitable combination. Accordingly, the embodiments are not limited other than in accordance with the appended claims and their equivalents. Furthermore, various modifications and changes can be made within the scope of the appended claims.

Furthermore, in describing representative embodiments, the specification may have presented methods and/or processes as a specific sequence of steps. However, to the extent that the method or process does not depend on the particular order of steps described herein, the method or process should not be limited to the particular order of steps described. As one of ordinary skill in the art will appreciate, other sequences of steps are also possible. Accordingly, the particular order of steps set forth in the specification should not be construed as limiting the claims. Furthermore, the claims directed to the method and/or process should not be limited to performing their steps in the order in which they are written, and those skilled in the art will readily appreciate that these orders may vary and still remain within the spirit and scope of the embodiments of the present application.

Experimental methods for which specific conditions are not specified in the following examples are usually measured according to national standards. The experimental materials whose source is not indicated in the following examples are all commercially available raw materials. The equipments used in each step in the following examples are conventional equipments. If there is no corresponding national standard, it will be carried out according to the general international standards, conventional conditions, or the conditions recommended by the manufacturer. Unless otherwise defined or stated, all technical and scientific terms used herein have the same meanings as those familiar to those skilled in the art. In addition, any methods and materials similar or equivalent to the described contents can be used in the methods of the present application.

Example 1. Obtaining and Identification of the *Lactiplantibacillus plantarum* JN-7

The *Lactiplantibacillus plantarum* JN-7 provided in the present application was isolated from homemade pickles in Chengdu, Sichuan, China.

1. 16s sequencing of the *Lactiplantibacillus plantarum* JN-7

JN-7 bacterial genomic DNA was extracted using the Takara Mini-BEST Bacterial Genomic DNA Extraction Kit version 3.0. The 16S rRNA gene was amplified by universal primers 27F: 5'-AGAGTTTTGATCCTGTCCAG-3' (SEQ ID NO: 1) and 1492R: 5'-GGTTACCTTGTTACGACTT-3' (SEQ ID NO: 2). PCR cycles: initial activation at 94 °C for 2 min; cycling steps: denaturation at 94 °C for 30 s; annealing at 55 °C for 1 min; extension at 72 °C for 1 minute; and finally at 72 °C for 10 minutes. The PCR product was purified for first-generation sequencing, and the 16s rRNA sequence measured is shown in SEQ ID NO: 3.

After aligning the 16s rRNA sequence of the *Lactiplantibacillus plantarum* by Blast on the NCBI website, it was found to have a similarity of 99.93% to that of the type strain *Lactiplantibacillus plantarum* SRCM100442. Based on the physiological and biochemical indicators and molecular biological identification results of strain JN-7, combined with its morphological characteristics, we named it as *Lactiplantibacillus plantarum* JN-7 and deposited it in China General Microbiological Culture Collection Center on May 13, 2024 under a Depositary Accession Number of CGMCC No. 30605.

2. Whole genome sequencing of the *Lactiplantibacillus plantarum* JN-7

For genome sequencing, the *Lactiplantibacillus plantarum* JN-7 was cultured under anaerobic conditions at 37 °C for 24 h using MRS broth. Single colony was cultured overnight in MRS broth. The broth was centrifuged at 8000 × g, 4 °C, 5 min. The precipitates were sent to GENEWIZ Sequencing Company (China) for sequencing, assembly, annotation, and bioinformatics analysis.

Whole genome sequencing was performed on the Illumina PE150 platform and the PacBio Sequel system. For the PacBio sequencing library, 5-10 μg of genomic DNA was sheared into fragments of 10-15 Kb using a g-TUBE device. A library was then constructed using SMRTbell® Express Template Preparation Kit 2.0. Briefly, DNA sheared fragments are subjected to single-strand overhang removal, DNA damage repair, end repair, A-tailing, and barcoded overhang adapter ligation. The library was quantified using a Qubit 3.0 fluorometer (Invitrogen, Carlsbad, Calif.) and the size of the library was checked using an Agilent 2100 bioanalyzer system. Follow the manufacturer's instructions for subsequent steps to prepare the SMRTbell library. The library

was sequenced using the PacBio Sequel platform. PacBio reads were assembled using Hifiasm/Canu. The genome was then recalibrated with previous Illumina data by using Pilon software. Prodigal/Augustus gene finding software has been used to find coding genes. Transfer RNA (tRNA) was detected in the genome using the program tRNAscan-SE with default parameter settings. rRNA was identified by using Barrnap. Other RNAs were identified by the rfam database. The coding genes were annotated by Diamond using the National Center for Biotechnology Information (NCBI) nr database. The function of the genes was then annotated by the GO (Gene Ontology) database and the pathway was annotated using the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. The proteins encoded by the genes were phylogenetically classified by the COG (Clusters of Orthologous Groups) database. Protein sequences with E < 1e-5 were searched in the CAZy database, Swiss_Prot database, Pfam database, CARD database, VFDB database or DFVF database using Diamond.

The genome sequence of the *Lactiplantibacillus plantarum* JN-7 was assembled and analyzed. The basic genomic information sequences are shown in Table 1. The genome length is 3255213 bp, with a total of 3218 genes and 3090 protein-coding genes. Create a genomic circle chart showing gene, ncRNA, GC content, and repeat sequence information, as seen in FIG. 1, using Circos (version 0.69) software.

Table 1. Basic genomic information of the *Lactiplantibacillus plantarum* JN-7

Feature	**Value**	**% of total**
Total genes	3218	100
Size (bp)	3255213	100
RNA genes	128	3.98
Protein-coding genes	3090	96.02
Protein-coding genes for enzymes	1058	32.88
Genes with transmembrane helix	850	26.41
Genes with signal peptide	162	5.03
Genes assigned to COGs	2328	72.34
G + C content (bp)	1450568	44.56

COG cluster	1264	54.3

3. Identification of the *Lactiplantibacillus plantarum* JN-7

The identification of the *Lactiplantibacillus plantarum* JN-7 was performed by calculating the average nucleotide identity (ANI) between the JN-7 genome sequence and the *Lactiplantibacillus plantarum* SRCM100442 standard strain genome sequence using an online ANI calculator (http://enve-omics.ce.gatech.edu/ani/). The ANI calculator uses the best hit (one-way ANI) and the reciprocal best hit (two-way ANI) between the two genomic datasets to estimate the average nucleotide identity. By comparing the JN-7 genome sequence with the *Lactiplantibacillus plantarum* reference strain genome sequence, an ANI result of 99.21% was obtained, indicating that the JN-7 strain belongs to the *Lactiplantibacillus plantarum* species.

A database of 1215 genomes of *Lactiplantibacillus plantarum* (retrieved on February 20, 2024) selected from NCBI was established using Makeblastdb software. The criteria for *Lactiplantibacillus plantarum* screening are complete genomes or draft genomes that have been uploaded to NCBI. The assembled JN-7 genome was compared to the *Lactiplantibacillus plantarum* genome database by Blastn, generating mismatched regions and the number of mismatched regions in the comparison entries. No references were found that were completely identical to the assembly result or allowed up to 1000 mismatches, indicating that the *Lactiplantibacillus plantarum* JN-7 was a strain that had never been reported in NCBI.

Example 2. Characterization of the *Lactiplantibacillus plantarum* JN-7

The features of the *Lactiplantibacillus plantarum* JN-7

The *Lactiplantibacillus plantarum* JN-7 was fermented and cultured, and the following features of the strain were measured.

1) Growth curve of the *Lactiplantibacillus plantarum* JN-7

The growth curve of JN-7 was plotted using a Growth profiler 960 (Enzyscreen B.V., Heemstede, Netherlands) at a temperature of 37 °C. The specific procedure is as follows: after 16 h of MRS liquid culture of JN-7, the bacteria were inoculated into fresh MRS broth and the final OD600 was adjusted to 0.1. Add the bacterial liquid with adjusted OD to a sterile 96-well plate, ensuring that the addition amount per well is 250 μL. A total of 11 parallel sets of

experiments (A1-A11) were performed. The bacteria were cultured under aerobic conditions at 200 rpm and 37°C for 48 hours, and the OD values were collected every 30 minutes.

As shown in Figure 2, a typical quadratic curve was fitted by Growth Profiler 960. Cultured at 37 °C, 200 rpm, the *Lactiplantibacillus plantarum* JN-7 with an initial OD of 0.1 reached an exponential growth phase within 18 hours and was in a stationary phase for the next 48 hours.

2) Acid, bile salt and gastrointestinal tract resistance

The fresh bacterial liquid cultured in MRS broth for 16 hours was centrifuged at 12,000 rpm and 4 °C for 2 minutes, and the precipitate was retained. The precipitate was washed twice with sterile PBS buffer, resuspended in sterile PBS (pH = 7) and the cell concentration was adjusted to 10^8 CFU/mL. This bacterial liquid was inoculated in MRS (N0), MRS at pH 3.0 (N1), or MRS liquid medium containing 0.3% bile salt (w/v, Sigma, USA) (N1) at an addition amount of 5%. After placing at 37 °C for 4 h, the viable cells of the *Lactiplantibacillus plantarum* JN-7 in different media were counted.

To test the gastrointestinal tract resistance of *Lactiplantibacillus plantarum* JN-7, fresh single colony was picked and inoculated into MRS broth and cultured at 37 °C for 16 h. Take 5mL of bacterial liquid and centrifuge under a condition of 12000 rpm and 4 °C for 2 minutes. The precipitate was washed with sterile PBS (pH = 7) and resuspended to a bacteria concentration of 10^7 CFU/mL. 100 μL of the bacterial suspension was added to 900 μL of simulated gastric fluid, which is consisted of 125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$, and 3 g/L pepsin (Sigma, USA), and the pH of which was adjusted to 3.0 with HCl. After incubation at 37 °C for 3 hours, 250 μL of gastric fluid mixture was added to 6 mL of simulated intestinal fluid, and then incubated at 37 °C for 3 hours. The simulated intestinal fluid is consisted of 45 mM NaCl, 1 g/L trypsin (Sigma, USA), and 3 g/L bile salts (Sigma, USA), and its pH is adjusted to 8.0 by NaOH.

The survival rate of the *Lactiplantibacillus plantarum* JN-7 under acid, bile salt and gastrointestinal fluids treatment was calculated as follows:

Survival rate (%) = (N1 ÷ N0) × 100%

Herein, N1 is the viable cell count after treatment with MRS (pH 3.0, 0.3% bile salt) or gastrointestinal fluid for 6 hours; N0 is the viable cell count after treatment with MRS and gastrointestinal fluid for 0 hours.

The results showed that the survival rates of the *Lactiplantibacillus plantarum* JN-7 were 28.47% and 44.68% in the MRS at pH 3.0 and in the MRS with 0.3% bile salt, respectively. The viability of JN-7 in gastrointestinal fluid was evaluated in simulated gastric fluid for 3 h and then in simulated intestinal fluid for 3 h. After 6 h of treatment, the number of viable cells of JN-7 was decreased to 12.32% of that before treatment.

3) Sensitivity to antibiotics and MIC

Seven antibiotics commonly used in clinic, gentamicin, ampicillin, kanamycin, chloramphenicol, tetracycline, erythromycin and clindamycin, were selected to analyze the antibiotic sensitivity of the strains. 256 mg/L antibiotic stock solution was prepared and diluted in 1:2, and the fresh bacterial liquid after 16 h of culture was diluted to OD 0.0002, and finally inoculated into the above mentioned antibiotic solutions of different concentrations at a 1:1 inoculum ratio. The results were observed after 24 hours of culture at 37 ℃, and the minimum inhibitory concentration (MIC) was determined. The antibiotic resistance standards of bacteria formulated by the European Food Safety Authority (EFSA) were used as the standard for comparative evaluation.

The MICs of ampicillin, gentamicin, kanamycin, erythromycin, clindamycin, tetracycline and chloramphenicol against the *Lactiplantibacillus plantarum* JN-7 are shown in Table 2. The results showed that the MIC values of all seven antibiotics were lower than the antibiotic critical values specified in the EFSA guidelines, indicating that the strain was not resistant to antibiotics and had antibiotic safety.

Table 2. MIC of different antibiotics against the *Lactiplantibacillus plantarum* JN-7 (mg/L)

Ampicillin	Gentamicin	Kanamycin	Erythromycin	Clindamycin	Tetracycline	Chloramphenicol
1	8	64	1	1	16	4

4) Hemolytic activity

The absence of hemolytic activity and antibiotic resistance are considered safe prerequisites for the selection of probiotic strains (FAO/WHO, 2002).

The *Lactiplantibacillus plantarum* JN-7 was activated, and after two generations, was streaked in Columbia blood agar medium (3400071, Haibo Co., China) containing 5% defibrinated sheep blood and cultured at 37 °C for 48 hours. If a grass green hemolytic ring

appeared, it is α-hemolysis. If a colorless and transparent hemolytic ring appears, it is β-hemolysis. If there is no hemolytic ring, it is γ-hemolysis.

Results were shown in FIG. 3. After the *Lactiplantibacillus plantarum* JN-7 was cultured on blood agar, no hemolytic ring appeared around the colony, which was γ-hemolysis. This shows that the *Lactiplantibacillus plantarum* JN-7 has no hemolytic ability.

5) Production of D-lactate and L-lactate and analysis of L-lactate/D-lactate ratio

Fresh single colony was picked and cultured in MRS broth at 37 °C for 18 hours. Culture supernatants were subjected to D,L-lactate analysis by enzymatic method using a commercial D,L-lactate quantification kit (Jingmei Co., China) according to the manufacturer's protocol.

The production of D-lactate and L-lactate by the *Lactiplantibacillus plantarum* JN-7 was analyzed after culturing the strain in MRS broth at 37°C for 18 h. 1.2 nmol/L D-lactate and 4.28 nmol/L L-lactate were produced during JN-7 fermentation. The L-lactate/D-lactate ratio is 3.57, as seen in Table 3.

Table 3. D-lactate, L-lactate content and L-lactate/D-lactate ratio

Name	**D-lactate (nmol/L)**	**L-lactate (nmol/L)**	**L/D lactate ratio**
JN-7	1.20	4.28	3.57

6) Biogenic amine detection

The strain was cultured in MRS liquid medium overnight. The bacteria were then added to MRS liquid medium containing 0.1 g/L histidine, tyrosine, ornithine, lysine, and 0.05 g/L pyridoxal-5-phosphate to a final concentration of OD 0.01 and passaged every 24 h for a total of five times. The above liquid culture medium was inoculated into MRS medium containing 1% histamine, tyrosine, cadaverine and putrescine at a 2% inoculum amount and cultured for 72 h, and the color change of the culture medium was observed. If the medium color turns purple, it is positive, and if the medium turns yellow, it is negative. *Lactobacillus rhamnosus* LGG was used as a negative control and *Escherichia coli* was used as a positive control.

The results showed that JN-7 showed yellow color after three days of growth in four biogenic amine detection media, which indicated that the strain did not produce putrescine, cadaverine, histamine and tyramine.

7) API 50CH Sugar fermentation experiment

The fresh bacterial liquid cultured for 16 hours was centrifuged at 12000 rpm for 2 minutes. The supernatant was discarded, and the precipitate was washed twice with sterile PBS, then resuspended in sterile PBS, and the OD was adjusted to 0.3 to obtain bacterial liquid a. Take 1 mL of bacterial liquid a to 10 mL of 50CHL medium (50410, API, France) to obtain bacterial liquid b. 150μL of bacterial liquid b was sequentially added to the test wells of 50CH test strips (50300, API, France), and cultured at 37 ℃ for 48 h. The result was judged by the color change, and the color of yellow compared with the control group represented positive (wherein esculin positive was purple), and no change was recorded as negative.

The results showed that *Lactiplantibacillus plantarum* JN-7 can metabolize D-ribose (5), D-galactose (10), D-glucose (11), D-fructose (12), D-mannitol (13), mannitol (18), sorbitol (19), methyl-α-D-mannopyranoside (20), N-acetylglucosamine (22), amygdalin (23), arbutin (24), esculin (25), salicin (26), D-cellobiose (27), D-maltose (28), D-lactose (29), D-melobiose (30), D-sucrose (31), D-trehalose (32), D-melezitose (34), D-raffinose (35), D-gentiobiose (39), D-toulonose (40), D-tagatose (42) and potassium gluconate (47) to produce acid through fermentation..

Example 3. Cytotoxicity and adhesion of the *Lactiplantibacillus plantarum* JN-7

Experimental Method:

Culture of HT-29 cells

HT-29 is a human intestinal epithelial cell line used to test the cytotoxicity and adhesion of bacteria. HT-29 was cultured in RPMI 1640 medium containing 10% FBS and 1% P/S (penicillin/streptomycin). Culture in a 37 ℃ incubator containing 5% CO_2 for about 36h-72h. When the cell density reaches about 90%, digest with trypsin, passage, and inoculate plates.

Cell viability assay

CCK-8 method is a universal method to detect cell proliferation and toxicity. In this experiment, CCK-8 kit (C0037, Biyuntian) was used to detect the proliferation activity of HT-29 cells. The cells were counted and seeded into a 96-well plate at an appropriate density. CCK-8 solution was added in 1/10 volume of the total volume, and incubated at 37°C for 2 hours in the

dark. The bubbles were removed and the OD value at 450 nm was measured using a microplate reader.

Adhesion test

HT-29 cells were inoculated from a culture flask into a 24-well plate at a concentration of 5 × 10^5 cells/mL, and cultured in a medium without antibiotics. The experiment could be performed after the cells were completely attached. Prior to the addition of bacteria, the cells in the well plate were washed twice with sterile PBS, and 500 μL of bacteria at a concentration of 10^8 CFU/mL (V_0) was then added to each well. The 24-well plate was transferred to a 5% CO_2, 37 °C incubator for 4 h to adhere. Cells in each well were washed 5 times with PBS solution to elute non-adherent bacteria and metabolic secretions. 200 μL of 1% Triton X-100 was added to each well for digestion, and then the solution in each well was collected for gradient dilution and counted (V_1). The adhesion ratio (%) was calculated as follows:

Adhesion ratio (%) = (V_1/V_0)×100%

To evaluate the potential cytotoxic effect of the *Lactiplantibacillus plantarum* JN-7 on HT-29 intestinal epithelial cells, CCK-8 assays were performed after 18 h of co-culture of the *Lactiplantibacillus plantarum* JN-7 bacterial cells with the HT-29 cells. The Multiplicity of infection (MOI) of this test was about 1:200. As shown in a of FIG. 4, the *Lactiplantibacillus plantarum* JN-7 had no effect on the survival of intestinal epithelial cells at this MOI. The adhesion capacity of the *Lactiplantibacillus plantarum* JN-7 was compared to that of the commercial probiotic *Lactobacillus rhamnosus* (LGG) (b in FIG. 4). The results showed that LGG adhered to cells twice as efficiently as JN-7.

Example 4. Anti-inflammatory effect of the *Lactiplantibacillus plantarum* JN-7

Experimental Method:

Culture and treatment of THP-1

THP-1 is a kind of human monocytic leukemia cell, which can differentiate into macrophages under the induction of phorbol ester (PMA), and is used to detect the anti-inflammatory effect of bacteria. THP-1 was cultured in RPMI 1640 medium containing 10% FBS and 1% P/S. Culture in a 37°C incubator containing 5% CO_2. Passage when the cell density

reaches 8-10×10^5 cells/mL. The normal state of cells is suspension growth and can be passaged by half medium replacement.

THP-1 cells were seeded at 5 × 10^5 cells/mL in 6-well plates and cultured overnight in a CO_2 incubator at 37 °C. PMA was added at a concentration of 100 ng/mL to induce cell differentiation. After 48 h, the cells attached took on a spindle-shaped or polygonal shape, indicating differentiation into macrophages.

Real-time fluorescent quantitative PCR (qRT-PCR)

Total mRNA was isolated from cells using Trizol reagent (R701-01-AA, Vazyme) according to the manufacturer's instructions. Reverse transcription PCR (RT-PCR) was performed using HiScript II select qRT supermix (R222-01, Vazyme) in a final volume of 20 μL containing 1 μg RNA. qRT-PCR was performed on a quantitative Real-time PCR (qRT-PCR) detection system using gene-specific primers and SYBR Green (Q712-02, Vazyme). All primers were designed by ourselves and synthesized at GENEWIZ Biotechnology Co., Ltd. (Beijing, China). β-actin was used as the reference gene and the 2-ΔΔt method was used for data analysis.

The specific operation steps are as follows:

RNA extraction: (1) Collect the cells in a 1.5 mL enzyme-free EP tube, add 200 μL of Trizol reagent, and homogenate to fully lyse the cells. Incubate at room temperature for 5 min, then centrifuge at 12000 rpm at 4 °C for 5 min. (2) Transfer the supernatant to a new EP tube, add 4/5 volume of isopropyl alcohol, shake upside down for 10 times to mix thoroughly, and stand at room temperature for 10 min. (3) Centrifuge at 12000 rpm at 4 °C for 10 min, discard the supernatant, and a white feather-like precipitate appears, which is RNA. Wash twice with 800μL DEPC water containing 75% ethanol, discard the supernatant, evaporate the precipitate, and dissolve the precipitate with 20μL DEPC water on ice to obtain RNA. (4) Determine the concentration of RNA using a NanoDrop Microvolume spectrophotometer.

Synthesis of cDNA by RT-PCR: cDNA was synthesized using the RT-PCR kit from Vazyme (HiScript II Q RT SuperMix for qPCR).

Table 4. Preparation of first strand cDNA synthesis reaction solution

Reagent	Volume

RNase-free ddH_2O	to 20 μL
5 × HiScript II qRT SuperMix	4 μL
Total RNA	1 μg/quantitative concentration

After gently mixing, centrifuge at 500 rpm for 1min to the bottom.

Table 5. The first strand cDNA synthesis reaction was carried out under the following conditions

50℃	15 min
85℃	5 sec

20 μL of the resulting product can be immediately used in the qRT-PCR reaction or stored at -20 °C (available within half a year). For long-term storage, it is recommended to divide the samples into aliquots and store the aliquots at -80°C, and avoid repeated freezing and thawing.

qRT-PCR:

Table 6. A total of 20 μL of the reaction system is as follows:

Reagent	**Volume**
2 × Taq Pro SYBR Green PCR Mix	10 μL
Forward primer (10 μM)	0.4 μL
Reverse primer (10 μM)	0.4 μL
cDNA	1 μL
ddH_2O	8.2 μL

After gently flicking to mix, centrifuge the liquid to the bottom and amplify on a real-time fluorescence quantitative PCR instrument.

The reaction conditions were 94 °C for 30 s; 94 °C for 5 s; 60 °C for 30 s and repeated for 40 cycles. Bio-Rad CFX Manager Software automatically recorded the amplification data and melting curves. Amplification data are expressed as threshold cycles (CT values), and the data was analyzed using the 2-ΔΔt method. The primer sequences (SEQ ID NOs: 4-11) are shown in the table below.

Table 7. Primer Sequences

Gene Name	Upstream primer sequences (5'-3')	Downstream primer sequences (5'-3')
IL-6	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
IL-8	GAGAGTGATTGAGATGGACCAC	CACAACCCTCTGCACCCCAGTTT
IL-10	TCTCCGAGATGCCTTCAGCAGA	TCAGACAAGGCTTGGCAACCCA
β-actin	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT

Anti-inflammatory effect of JN-7 on LPS-stimulated PMA-differentiated THP-1 cells

JN-7 down-regulated the mRNA expression of pro-inflammatory factor in cells stimulated by LPS (as shown in FIG 5). JN-7 and LGG were co-cultured with macrophages differentiated from THP-1, respectively, and the anti-inflammatory effects of the strains were evaluated by detecting the mRNA expression levels of pro-inflammatory factors IL-6, IL-8 and anti-inflammatory cytokine IL-10. Compared to the LPS-stimulated control group, cells treated with JN-7 and LGG after LPS stimulation showed decreased IL-6 and IL-8 mRNA expression and increased IL-10 mRNA expression. Furthermore, LGG was more effective than JN-7 in downregulating LPS-stimulated IL-8 mRNA expression. The ability of JN-7 to promote IL-10 release is higher than that of LGG, suggesting that like LGG, JN-7 also has anti-inflammatory effects.

Example 5. Production and stability analysis of the *Lactiplantibacillus plantarum* JN-7 powder

1) JN-7 fermentation and bacterial powder production

The *Lactiplantibacillus plantarum* JN-7 was inoculated into MRS broth, cultured at 37 °C for 18 h. The cells were activated twice and then cultured in a 5-L bioreactor (BioFlo® 320, Eppendorf) for 12 hours. After fermentation, the bacteria were collected by centrifuging at 4 °C, 10000 rpm for 10 min. The obtained bacteria are mixed with protectant (1-10 g/L polysaccharide, 20-50 g/L disaccharide, 1-20 g/L vitamin C or its salt substance, and 1-10 g/L peptone), and pre-frozen at -80°C for 24 hours. Freeze-dry in a vacuum freeze-dryer (PO14416, Telstar LyoQuest-55plus) and pulverize into powder to obtain a bacterial powder, and then vacuum-pack the

bacterial powder into an aluminum foil bag.

After 12 hours of fermentation in a 5L bioreactor and centrifugation, a total of 41.5g of *Lactiplantibacillus plantarum* precipitate was collected. The precipitate was mixed with the protectant and the mixture was freeze-dried to yield 6.6 g of bacterial powder with a viable cell number of 7.07 x 10^{11} CFU/g.

2) Evaluation of stability of the *Lactiplantibacillus plantarum* JN-7 bacterial powder

The packed *Lactiplantibacillus plantarum*, i.e. the *Lactiplantibacillus plantarum* JN-7 bacterial powder packed into aluminum foil bags were stored in a constant humidity chamber at 40°C and 75% humidity for accelerated test. At the same time, the commercial *Lactobacillus rhamnosus* LGG bacterial powder and 15 strains of *Lactiplantibacillus plantarum* isolated from fermented vegetables were stored at the same temperature and humidity as controls. Within 90 days, samples of JN-7 bacterial powder, LGG bacterial powder, and the 15 strains of *Lactobacillus plantarum* bacterial powders were sampled to detect the number of bacteria in the bacterial powders and determine the water activity according to the China national standard GB 4789.35.

Viable cell counts and water activity of the *Lactiplantibacillus plantarum* JN-7 (FIG. 6A), the *Lactobacillus rhamnosus* LGG (FIG. 6B) and 15 strains of other *Lactiplantibacillus plantarum* bacterial powders (FIG. 6C) during storage are shown in FIGS. 6A-6C. It can be seen from the figures that the stability of JN-7 bacterial powder is significantly higher than that of the other 16 bacterial powders. The viable cells of JN-7 decreased from 7.07×10^{11} to 1.73×10^{11} CFU/g within 70 days, and the corresponding water activity increased from 0.024 to 0.053. The viable cells of control LGG decreased from 3.53×10^{11} to 3.92×10^{8} CFU/g within 70 days, and the corresponding water activity increased from 0.091 to 0.134. A linear fit ($r^2 = 0.80$) was performed for the reduction of JN-7 viable bacteria over 70 days of testing. Through fitting, it can be predicted that under accelerated stability test conditions, the time required to reduce the number of viable bacteria by 1 log is 136.99 days, which is 112.36 days longer than the 24.63 days required for LGG predicted by fitting.

Overall, the present application isolated and identified a *Lactiplantibacillus plantarum* JN-7 from a naturally fermented product. After whole genome sequencing and comparison with all

NCBI-retrievable *Lactiplantibacillus plantarum* genomes, we found that the *Lactiplantibacillus plantarum* JN-7 strain was a novel strain. Through the analysis of basic characteristics (growth curve, acid resistance, bile salt resistance, gastrointestinal fluid resistance), safety evaluation (antibiotic sensitivity, hemolytic ability, toxigenic ability, cytotoxicity), adhesion evaluation, anti-inflammatory effect evaluation, production performance evaluation (fermentation yield and freeze-drying yield) and stability test (accelerated storage stability test) of the strain, the *Lactiplantibacillus plantarum* JN-7 was found to be a safe strain with excellent production performance, high stability and anti-inflammatory efficacy.

Claims

1. A *Lactiplantibacillus plantarum* JN-7, wherein the *Lactiplantibacillus plantarum* JN-7 has a taxonomic designation of *Lactiplantibacillus plantarum*, and a Depositary Accession Number of CGMCC No. 30605.

2. A bacterial powder prepared from the *Lactiplantibacillus plantarum* JN-7 of claim 1.

3. A method for preparing the bacterial powder of claim 2, comprising the following steps:

1) fermenting the *Lactiplantibacillus plantarum* JN-7;

2) centrifuging the fermentation product to obtain a precipitate, and freeze-drying the precipitate to obtain the bacterial powder.

4. A probiotic composition comprising the *Lactiplantibacillus plantarum* JN-7 of claim 1 or the bacterial powder of claim 2.

5. The probiotic composition according to claim 4, wherein the probiotic composition further comprises one or more probiotics selected from: *Bifidobacterium, Lactobacillus, Lacticaseibacillus, Limosilactobacillus, Lactiplantibacillus, Ligilactobacillus, Latilactobacillus, Streptococcus, Lactococcus, Propionibacterium, Acidipropionibacterium, Weizmannia, Mammaliicoccus, Staphylococcus, Kluyveromyces, Leuconostoc, Pediococcus* and *Bacillus subtilis* DE111.

6. Use of the *Lactiplantibacillus plantarum* JN-7 of claim 1, the bacterial powder of claim 2 or the probiotic composition of claim 4 or 5 in the preparation of an anti-inflammatory medicament.

7. The use according to claim 6, wherein the medicament is administered orally.

8. Use of the *Lactiplantibacillus plantarum* JN-7 of claim 1, the bacterial powder of claim 2 or the probiotic composition of claim 4 or 5 in the preparation of foods or nutraceuticals.

9. The use according to claim 8, wherein the foods or nutraceuticals are dairy products, soy products, meat products, fruits and vegetable products, beverages, or snacks.

10. The use according to claim 8, wherein the foods or nutraceuticals further comprise edible adjuvants.