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Lactiplantibacillus Plantarum JN9, Formulation, Probiotic Composition and Uses Thereof

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ABSTRACT

A *Lactiplantibacillus plantarum* JN9, a formulation, a probiotic composition and uses thereof. The present application obtains a new strain of *Lactiplantibacillus plantarum* JN9 through screening, which has a Depositary Accession Number of CCTCC NO: M 20241647. Through the analysis of the basic characteristics (growth curve, acid resistance, bile salt resistance, gastrointestinal fluid resistance), safety evaluation (antibiotic sensitivity, hemolytic ability, toxin production ability, cytotoxicity, biogenic amines production ability), adhesion evaluation, GABA production evaluation, alcohol resistance and acetaldehyde dehydrogenase production ability evaluation of the strain, it was found to be a safe strain with excellent GABA production performance, high alcohol resistance and high acetaldehyde dehydrogenase activity. It can be used in products for improving mood, such as reducing stress, resisting depression, and improving sleep quality, lowering blood pressure, anti-diabetes, anti-cancer, anti-oxidation, anti-inflammation, anti-microbial, anti-allergy, as well as enhancing alcohol resistance during drinking and relieving discomfort after drinking, etc.

Lactiplantibacillus Plantarum JN9, Formulation, Probiotic Composition and Uses Thereof

Technical Field

The present invention relates generally to the field of biological application technology, in particular to a *Lactiplantibacillus plantarum* JN9, a formulation, a probiotic composition and uses thereof.

Background

γ-Aminobutyric Acid (abbreviated as GABA) is a non-protein natural amino acid, widely found in animals, plants and microorganisms. GABA is an important central nervous system inhibitory neurotransmitter that can modulate diseases associated with depression and other mental health problems. Studies have shown that taking a certain amount of GABA has physiological benefits such as improving the body's sleep, lowering blood pressure, treating epilepsy and regulating mood. Animal and clinical population trials have also confirmed that GABA has the curative effect of improving depression-like behavior in mice and alleviating depression in people. With the deepening of research, GABA has developed into a new functional factor, which is widely used in medicine, food health care, agriculture and other industries.

GABA can be prepared by chemical synthesis or biosynthesis, and the biosynthesis method of GABA may be more promising than the chemical synthesis method due to the characteristics of simple reaction steps, high catalytic efficiency, mild reaction conditions and good environmental compatibility, etc.

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 immune function of the host mucosa and system or regulating the balance of intestinal flora,

thus producing single microorganism or mixed microorganisms with clear composition that are beneficial to health. Finding a probiotic strain with high safety and ability to produce GABA is of great significance for improving mood, resisting depression and improving sleep quality.

Summary

Based on the above background, the present application provides a *Lactiplantibacillus plantarum* JN9, wherein the *Lactiplantibacillus plantarum* JN9 has a taxonomic designation of *Lactiplantibacillus plantarum*, and a Depositary Accession Number of CCTCC NO: M 20241647.

In another aspect, the present application also provides a formulation prepared from the *Lactiplantibacillus plantarum* JN9 described herein.

In another aspect, the present application also provides a probiotic composition comprising the *Lactiplantibacillus plantarum* JN9 described herein or the formulation described herein.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN9 described herein, the formulation described herein, or the probiotic composition described herein in the preparation of a medicament for reducing stress, improving mood, resisting depression, improving sleep quality, and/or lowering blood pressure.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN9 described herein, the formulation described herein, or the probiotic composition described herein in the preparation of a medicament for protecting liver and/or preventing alcohol hangover.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN9 described herein, the formulation described herein, or the probiotic composition described herein in the preparation of foods or nutraceuticals.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN9 described herein in the production of GABA.

The present application provides a *Lactiplantibacillus plantarum* strain which can produce GABA, tolerate alcohol, and has good safety, acid, bile salt, gastrointestinal tract resistance, and antibiotic sensitivity, and is designated as *Lactiplantibacillus plantarum* JN9. It was deposited at: China Center for Type Culture Collection, Address: No. 299, Bayi Road, Wuchang District, Wuhan City, Hubei Province, on a depositary date of July 22, 2024, and under a Depositary Accession Number of CCTCC NO: M20241647.

Through the analysis of the basic characteristics (growth curve, acid resistance, bile salt resistance, gastrointestinal fluid resistance), safety evaluation (antibiotic sensitivity, hemolytic ability, toxin production ability, cytotoxicity, biogenic amines production ability), adhesion evaluation, GABA production evaluation, alcohol resistance and acetaldehyde dehydrogenase production ability evaluation of *Lactiplantibacillus plantarum* JN9, it was found that the strain was a safe strain with excellent GABA production performance, high alcohol resistance and high acetaldehyde dehydrogenase activity. Based on the efficacy of the produced GABA, strain JN9 can be used in products for improving mood, such as reducing stress, resisting depression and improving sleep quality of the body, lowering blood pressure, anti-diabetes, anti-cancer, anti-oxidation, anti-inflammatory, anti-microbial, and anti-allergy, etc. Based on the strong resistance of JN9 strain to alcohol and high acetaldehyde dehydrogenase enzyme activity, it can be used in products that enhance alcohol resistance during drinking and alleviate discomfort after drinking.

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* JN9 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, the fourth circle is coding genes on the negative strand, the fifth circle is ncRNA on the positive strand, the sixth circle is ncRNA information on the negative strand, and the seventh circle is long fragment repeat information on the genome.

FIG. 2 shows colony morphology and Gram staining results of the *Lactiplantibacillus plantarum* JN9 in examples of the present application. Herein, (a) is the morphology of the *Lactiplantibacillus plantarum* JN9 colony, and (b) is the morphology of the *Lactiplantibacillus plantarum* JN9 body.

FIG. 3 is an API 50CH sugar fermentation test result of *Lactiplantibacillus plantarum* JN9 in an example of the present application.

FIG. 4 is a growth curve of the *Lactiplantibacillus plantarum* JN9 over 48 h in an example of the present application.

FIG. 5 is an assessment of resistance of the *Lactiplantibacillus plantarum* JN9 in an example of the present application. Herein, (a) is the survival rate of the *Lactiplantibacillus plantarum* JN9 in MRS with pH = 3.0 or 0.3% bile salt after 3 h; and (b) is the survival rate of the *Lactiplantibacillus plantarum* JN9 in sterile electrolyte solution containing 100 mg/L lysozyme for 30 min and 1 h.

FIG. 6 is a graph showing hemolysis results of the *Lactiplantibacillus plantarum* JN9 in

an example of the present application.

FIG. 7 is a graph showing the results of cytotoxicity of the *Lactiplantibacillus plantarum* JN9 to intestinal epithelial cell HT-29 in an example of the present application. Data are expressed as Mean ± Sem., n = 6.

FIG. 8 is a graph showing the results of cell adhesiveness of the *Lactiplantibacillus plantarum* JN9 in an example of the present application.

FIG. 9 is a graph showing the results of the GABA production capacity of the *Lactiplantibacillus plantarum* JN9 in an example of the present application. Herein, (a) is a PCR amplification map of *gadB*; and (b) is a mass spectrum of a GABA standard sample and a fermentation broth of the *Lactiplantibacillus plantarum* JN9.

FIG. 10 shows detection of the acetaldehyde dehydrogenase gene (*aldh*) and the alcohol dehydrogenase gene (*adh*) of the *Lactiplantibacillus plantarum* JN9 in an example of the present application. Herein, (a) is an *adh* gene amplification map, and (b) is an *aldh* gene amplification map.

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 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.

At present, most of the probiotic products on the market focus on gastrointestinal regulation, women's physiological health, pet health, etc. There are no hot-selling probiotic products or leading brands focusing on emotional health, relieving anxiety and depression, etc. The present application develops strains with antidepressant effects for people suffering from emotional problems such as insomnia, anxiety, depression, etc., and screens out probiotics capable of converting monosodium glutamate, one of the commonly used condiments in people’s daily diet, into GABA. This provides the possibility of discovering antidepressant probiotics that improve sleep and supplements the combination of antidepressant probiotic products. In addition, the *Lactiplantibacillus plantarum* JN9 not only has high GABA transformation ability, but also has strong resistance to alcohol and high acetaldehyde dehydrogenase activity, which makes this strain not only have application potential in the market of improving sleep and anti-anxiety, but also has application potential in the market of alcohol resistance and alleviating discomfort after drinking.

The present application provides a *Lactiplantibacillus plantarum* JN9, wherein the *Lactiplantibacillus plantarum* JN9 has a taxonomic designation of *Lactiplantibacillus plantarum*, and a deposit number of CCTCC NO: M 20241647. This strain was isolated from fermented foods for the first time, and the whole genome sequencing showed that it was a

Lactiplantibacillus plantarum strain that was not reported in NCBI.

In another aspect, the present application also provides a formulation prepared from the *Lactiplantibacillus plantarum* JN9 described herein.

In some embodiments, the formulation is selected from one or more of:

1) a bacterial dry product produced from the *Lactiplantibacillus plantarum* JN9; 2) a bacterial liquid product produced from the *Lactiplantibacillus plantarum* JN9; 3) a postbiotic of the *Lactiplantibacillus plantarum* JN9; 4) metabolites of the *Lactiplantibacillus plantarum* JN9; and 5) fermented extracts of the *Lactiplantibacillus plantarum* JN9.

In some embodiments, the *Lactiplantibacillus plantarum* JN9 described herein is fermented and then made into various formulations described herein. Methods for preparing the various formulations described herein are known to those skilled in the art.

In another aspect, the present application also provides a probiotic composition comprising the *Lactiplantibacillus plantarum* JN9 described herein or the formulation 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*.

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* JN9 described herein, the formulation described herein, or the probiotic composition described herein in the preparation of a medicament for reducing stress, improving mood, resisting depression, improving sleep quality, and/or lowering blood pressure.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN9 described herein, the formulation described herein, or the probiotic composition described herein in the preparation of a medicament for protecting liver and/or preventing alcohol hangover.

In some embodiments, the medicament is administered orally.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN9 described herein, the formulation described herein, or the probiotic composition described herein in the preparation of foods or nutraceuticals.

In some embodiments, the foods or nutraceuticals are foods or nutraceuticals for reducing stress, improving mood, resisting depression, improving sleep quality, lowering blood pressure, and/or improving alcohol resistance.

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 includes 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* JN9 described herein and edible adjuvants.

In another aspect, the present application also provides the use of the *Lactiplantibacillus plantarum* JN9 described herein in the production of GABA.

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 *Lactiplantibacillus plantarum* JN9

The *Lactiplantibacillus plantarum* JN9 provided in the present application was isolated from a pickle from Sichuan, China.

1. 16s rRNA sequencing of the *Lactiplantibacillus plantarum* JN9

The *Lactiplantibacillus plantarum* JN9 was cultured at 37 °C for 16 h to collect fresh bacteria, and DNA was extracted by using a DNA extraction kit (9763, Takara, Japan). The genomic DNA was amplified by 16s rRNA PCR, purified and then sequenced. The 16s rRNA amplification primers were universal primers 27F: 5'-AGAGTTTGATCCTGTCCAG-3' (SEQ ID NO:1) and 1492R: 5'-GGTTACCTTGTTACGACTT-3' (SEQ ID NO:2). The PCR reaction conditions were denaturation at 94 °C for 5 min; 30 cycles in total of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, extension at 72 °C for 1 min; ,and extension at 72 °C for 10 min. The PCR product was purified for first-generation sequencing, and the 16s

rRNA sequence measured is shown in SEQ ID NO: 3.

After sequencing the 16s rRNA of the *Lactiplantibacillus plantarum* JN9, it was found to have a similarity of 99.86% to the standard strain SRCM100442 of *Lactiplantibacillus plantarum*. Combining the physiological and biochemical indexes, morphological characteristics and molecular biological identification results of the strain of the *Lactiplantibacillus plantarum* JN9, we named it as *Lactiplantibacillus plantarum* JN9, and deposited it in China Center for Type Culture Collection on July 22, 2024 under a Depositary Accession Number of CCTCC NO: M20241647.

2. Whole genome sequencing of the *Lactiplantibacillus plantarum* JN9

For genome sequencing, the *Lactiplantibacillus plantarum* JN9 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* JN9 was assembled and analyzed. The basic genomic information sequences are shown in Table 1. The genome length is 3187478 bp, with a total of 3151 genes and 3029 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* JN9

Feature	**Value**	**% of total**
Size (bp)	3187478	100
G + C content (bp)	1425066	44.71
Coding region (bp)	2687457	84.31
Total genes	3151	100
RNA genes	122	3.87
Protein-coding genes	3029	96.13
Protein-coding genes with enzymes	1032	32.75
Genes assigned to COGs	2318	73.56
COG Clusters	1247	53.8
Genes with signal peptides	163	5.17
Genes with transmembrane helix	831	26.37

3. Identification of the *Lactiplantibacillus plantarum* JN9

The average nucleotide identity (ANI) was estimated by calculating the best hit (one-way ANI) and mutual best hit (two-way ANI) between the *Lactiplantibacillus plantarum* JN9 genome sequence and the *Lactiplantibacillus plantarum* standard strain GCA_009913655.1

(ASM991365v1) using an online ANI calculator (http://enve-omics.ce.gatech.edu/ani/) to determine the genus and species of *Lactiplantibacillus plantarum* JN9.

A total of 1215 genome sequences of *Lactiplantibacillus plantarum* were obtained by querying the complete genome or genome draft of *Lactiplantibacillus plantarum* in NCBI (retrieved on February 20, 2024). A database of the 1215 genomes of *Lactiplantibacillus plantarum* was established using Makeblastdb software, and the complete genome of the *Lactiplantibacillus plantarum* JN9 was compared with the database by Blastn, and the mismatch regions and the number of mismatch regions in the comparison entries were generated according to 1000 mismatches.

The ANI value of the *Lactiplantibacillus plantarum* JN9 genome sequence and the *Lactiplantibacillus plantarum* reference strain GCA_009913655.1 obtained by the online ANI calculator was 99.10%, indicating that the *Lactiplantibacillus plantarum* JN9 was *Lactiplantibacillus plantarum*. At the same time, the genome of the *Lactiplantibacillus plantarum* JN9 was compared with genomes of 1215 strains of *Lactiplantibacillus plantarum* by Blastn, and no reference was found that was completely consistent with the assembly result or allowed up to 1000 mismatches, indicating that the *Lactiplantibacillus plantarum* JN9 was a strain that had never been reported in NCBI.

Example 2. Characterization of the *Lactiplantibacillus plantarum* JN9

The *Lactiplantibacillus plantarum* JN9 was fermented and cultured, and the following characteristics of the strain were measured

1) Morphological observation and sugar fermentation test of the *Lactiplantibacillus plantarum* JN9

The colony morphology of the *Lactiplantibacillus plantarum* JN9 was observed, and a single colony was selected for Gram staining and examined under microscope at 100X.

The sugar fermentation test of the *Lactiplantibacillus plantarum* JN9 was carried out by API 50CH (50300, API, France). Specific operations are as follows. Culture the *Lactiplantibacillus plantarum* JN9 at 37 °C for 16 h, and then collect fresh bacteria. Wash the

bacteria twice with sterile PBS, and then resuspend it in sterile PBS, and adjust the OD to 0.3. Add 1mL of resuspended bacterial liquid to 10mL of 50CHL medium. After mixing, add 150μL to 50CH test strip test wells, and culture at 37 °C for 48h. The result was judged by the color change, and a color of yellow compared with the control group represented as a positive result (wherein esculin hydrate positive was black-red), and no change was recorded as negative.

The results showed that the colony morphology of the *Lactiplantibacillus plantarum* JN9 grown on the MRS plate was milky white, round, with neat edges, a smooth and moist surface, and the colony diameter was about 1-2 mm ((a) in FIG. 2). Pick a single colony for Gram staining and then observe under a light microscope. It was found to be Gram-positive bacteria and showed a rod shape ((b) in FIG. 2).

The results of carbohydrate utilization by the *Lactiplantibacillus plantarum* JN9 are shown in FIG. 3: the *Lactiplantibacillus plantarum* JN9 can metabolize D-ribose (5), D-galactose (10), D-glucose (11), D-fructose (12), D-mannitol (13), mannitol (18), methyl-α-D-mannopyranoside (20), methyl-α-D-glucopyranoside (21), N-acetylglucosamine (22), amygdalin (23), arbutin (24), esculin hydrate (25), salicin (26), D-cellobiose (27), D-maltose (28), D-lactose (29), D-melibiose (30), D-sucrose (31), D-trehalose (32), D-melezitose (34), D-gentiobiose (39), D-toulose (40) and potassium gluconate (47) to produce acid through fermentation.

2) Growth curve measurement of the *Lactiplantibacillus plantarum* JN9

The growth curve of the *Lactiplantibacillus plantarum* JN9 was plotted using Growth profiler 960 (Enzyscreen B.V., Heemstede, Netherlands). The specific operations are as follows. The *Lactiplantibacillus plantarum* JN9 is cultured in MRS for 16 hours and then inoculated into fresh MRS broth, and the final OD600 is 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. Perform 11 parallel sets of experiments(A1-B9). 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. At the same time, MRS liquid medium was used as a blank control group.

The *Lactiplantibacillus plantarum* JN9 with an initial OD of 0.1 was cultured in MRS liquid medium at 37 °C, 200 rpm, reaching an exponential growth phase within 16 hours and being in a stable phase within the next 48 hours. FIG. 4 is a growth curve of the *Lactiplantibacillus plantarum* JN9 fitted by Growth Profiler 960.

3) Resistance of the *Lactiplantibacillus plantarum* JN9

Acid and bile salt resistance

The *Lactiplantibacillus plantarum* JN9 was cultured in MRS broth at 37 °C for 16 h, and the fresh bacterial liquid was centrifuged at 4 °C and 12000 rpm for 2 minutes to collect the bacteria. The bacteria were washed twice with sterile PBS buffer, resuspended in PBS (pH = 7) and the bacteria concentration was adjusted to 10^8 CFU/mL. The resuspended bacterial liquid was inoculated into MRS broth with pH = 3.0 or 0.3% bile salt (w/v, Sigma, USA) according to the addition amount of 5%, and incubated in a 37 °C incubator for 3 hours. The numbers of viable bacteria of the *Lactiplantibacillus plantarum* JN9 in different media before culture (N0) and after 3 h culture (N1) were recorded.

Lysozyme resistance

The *Lactiplantibacillus plantarum* JN9 was cultured in MRS broth at 37 °C for 16 h, and the fresh bacterial liquid was centrifuged at 4 °C and 12000 rpm for 2 minutes to collect the bacteria. The bacteria were washed twice with sterile PBS buffer and resuspended in 2 mL Ringer's solution (8.5 g/L NaCl, 0.4 g/L KCl, 0.34 g/L hydrated $CaCl_2$) to 10^8–10^9CFU/mL. The bacterial suspension was inoculated in a sterile electrolyte solution (SES) (0.22 g/L $CaCl_2$, 6.2 g/L NaCl, 2.2 g/L KCl, 1.2 g/L $NaHCO_3$). Lysozyme (Sigma, USA) was added to a final concentration of 100 mg/L and incubated at 37 °C for 30 minutes or 1 hour, and a bacterial suspension without lysozyme added in SES was used as a negative control. The numbers of viable *Lactiplantibacillus plantarum* JN9 before incubation (N0) and after incubation (N1) were recorded.

Gastrointestinal fluid resistance

In order to detect the gastrointestinal resistance of the *Lactiplantibacillus plantarum* JN9,

a fresh single colony was picked into MRS broth and cultured at 37 ℃ for 16 h. Take 5 mL of bacterial liquid and centrifuge at 12000 rpm and 4°C for 2 minutes. The bacteria were washed with sterile PBS (pH = 7) and resuspended, and the bacteria concentration was adjusted to 10^7 CFU/mL. Take 100 μL of bacterial suspension and add it to 900 μL of simulated gastric fluid (125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$ and 3 g/L pepsin, pH adjusted to 3.0 with HCl) and incubate at 37°C. After 3 hours, take 250 μL of the gastric fluid mixture and add it to 6 mL of simulated intestinal fluid (45 mM NaCl, 1 g/L trypsin, 3 g/L bile salts, pH adjusted to 8.0 with NaOH) and continue incubating at 37°C for 3 hours. The number of viable cells before incubation with gastric and intestinal fluids was recorded as N0, and the number of viable cells after 6 h of incubation multiplied by the dilution factor of 25 was recorded as N1.

The survival rate of the *Lactiplantibacillus plantarum* JN9 under acid, bile salt, lysozyme and gastrointestinal fluids treatment was calculated as follows:

Survival (%) = (N1 ÷ N0) × 100%

Wherein N1 is the viable cell count after 3 h in MRS (pH 3.0 or 0.3% bile salt), 30 min or 1 h in SES (100 mg/L lysozyme), or 6 h in gastrointestinal fluids. N0 is the viable cell count of the *Lactiplantibacillus plantarum* JN9 treated for 0 h under different conditions.

Resistance assessment of the *Lactiplantibacillus plantarum* JN9

The survival rates of the *Lactiplantibacillus plantarum* JN9 were 47.87% and 75.11% in the MRS at pH 3.0 and in the MRS containing 0.3% bile salt, respectively ((a) in FIG. 5). After 30 min and 1 h incubation in a sterile electrolyte solution containing 100 mg/L lysozyme, the survival rates were 87.18% and 73.42%, respectively ((b) in FIG. 5). After 6 hours of digestion in simulated gastric fluid and simulated intestinal fluid, the number of viable cells of the *Lactiplantibacillus plantarum* JN9 decreased to 12.50% of that before treatment.

4) Safety of the *Lactiplantibacillus plantarum* JN9

Sensitivity to antibiotics and MIC

Seven antibiotics commonly used in clinic, including gentamicin, ampicillin, kanamycin, chloramphenicol, tetracycline, erythromycin and clindamycin, were selected to analyze the

antibiotic sensitivity of the strains. Stock solutions containing 256 mg/L of different antibiotics were prepared and diluted in 1:2 series and added to a 96-well plate, with 100 μL per well. After 16 h of culture, the fresh bacterial liquid was diluted to OD 0.0002 and added to the antibiotic well plate at 100 μL/well and cultured at 37°C for 24 h. The minimum inhibitory concentration (MIC) of the *Lactiplantibacillus plantarum* JN9 for each antibiotic was determined and compared with the cut-off values of antibiotics recommended by the European Food Safety Authority (EFSA) for microorganisms that can be used as feed additives or in production.

The MICs of ampicillin, gentamicin, kanamycin, erythromycin, clindamycin, tetracycline and chloramphenicol against the *Lactiplantibacillus plantarum* JN9 are shown in Table 2. The results showed that the MIC values of the seven antibiotics tested were all lower than the antibiotic critical value specified in the EFSA guidelines, indicating that the strain was sensitive to these seven antibiotics and had microbial antibiotic safety.

Table 2 MIC of different antibiotics against the *Lactiplantibacillus plantarum* JN9 (mg/L)

Ampicillin	Gentamicin	Kanamycin	Erythromycin	Clindamycin	Tetracycline	Chloramphenicol
1	2	16	0.5	＜0.125	32	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* JN9 was activated, and after two generations, was streaked onto Columbia blood agar medium (3400071, Haibo, 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 appeared, it is β-hemolysis; if no hemolytic ring appeared, it is γ-hemolysis. At the same time, *Lactobacillus rhamnosus* LGG was used as a negative control.

Results were shown in FIG. 6. After the *Lactiplantibacillus plantarum* JN9 was cultured on blood agar, no hemolytic ring appeared around the colony, which was γ-hemolysis. This shows that the *Lactiplantibacillus plantarum* JN9 has no hemolytic ability.

Measurement of D-lactate and L-lactate production by the *Lactiplantibacillus plantarum* JN9

Fresh single colony was picked and cultured in MRS broth at 37 °C for 16 hours. The culture supernatant was analyzed for D/L-lactate by enzymatic method using a commercial D/L-lactate quantification kit (Jingmei Co., China) according to the product instructions.

The results showed that the concentrations of D-lactate and L-lactate in the supernatant were 2.28 nmol/L and 8.99 nmol/L, respectively, after the strain was cultured in MRS broth at 37 °C for 16 h. L-lactate/D-lactate was 3.94.

Biogenic amine production ability Test

The *Lactiplantibacillus plantarum* JN9 was activated and 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 at a 2% inoculum size every 24 h for a total of five times. The above liquid medium was inoculated into a culture medium containing 0.1% histidine, tyrosine, ornithine and lysine at a 2% inoculum size and cultured for 72 h. 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 the *Lactiplantibacillus plantarum* JN9 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.

Cytotoxicity

CCK-8 method is a universal method to detect cell proliferation and toxicity. HT-29 cells are human intestinal epithelial cell lines and were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% P/S (penicillin/streptomycin). Its cell proliferation activity was detected by Biyuntian CCK-8 kit. HT-29 cells were cultured in a 5% CO_2, 37°C incubator for about 36h-72h until the cell density reached about 90%. They were then digested

with trypsin and passaged, and seeded into 96-well plates at an appropriate density. After the cells were adhered, 100 μL of the *Lactiplantibacillus plantarum* JN9 at a concentration of 10^8 CFU/mL was added to each well and co-cultured with the cells for 18 h. Wash with sterile PBS to remove the cell culture medium and 1/10 of the total volume of CCK-8 solution was added. The cells were incubated in the dark at 37°C for 2 h. Remove bubbles and measure the absorbance at 450 nm using a microplate reader.

To evaluate the potential cytotoxic effect of the *Lactiplantibacillus plantarum* JN9 on HT-29 intestinal epithelial cells, CCK-8 assay was performed after 18 h of bacterial-cell co-culture. The Multiplicity of infection (MOI) of this test was about 1:200. As shown in FIG. 7, at this MOI, the effect of the *Lactiplantibacillus plantarum* JN9 on cell activity was not significantly different from that of the blank control group ($p>0.05$), indicating that the *Lactiplantibacillus plantarum* JN9 had no negative impact on the survival of HT-29 intestinal epithelial cells.

5) Intestinal adhesion of the *Lactiplantibacillus plantarum* JN9

HT-29 cells were inoculated from the 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 (V0) 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 (V1). The adhesion ratio (%) was calculated as follows:

$$\text{Adhesion ratio (\%)} = (V1/V0) \times 100\%$$

To evaluate the cell adhesion of the *Lactiplantibacillus plantarum* JN9 to HT-29 intestinal epithelial cells, an adhesion assay was performed after 4 h of bacterial-cell co-culture. The Multiplicity of infection (MOI) of this test was about 1:200. The results showed that the proportion of the *Lactiplantibacillus plantarum* JN9 that could adhere to HT-29 cells after 4 h

of co-culture was 15.98%, and its adhesion efficiency was approximately 1.5 times that of LGG (10.50%) (FIG. 8).

Example 3. Fermentation of the *Lactiplantibacillus plantarum* JN9 and production of bacterial powder

The *Lactiplantibacillus plantarum* JN9 was activated and then inoculated into MRS broth, cultured at 37 ℃ for 18h, transferred twice and then transferred into bioreactor (BioFlo ® 320, Eppendorf) for fermentation. The total amount of fermentation liquid was 6L. After 12 h of fermentation, the bacteria were collected at 4 °C, 10000 rpm, and 10 min. The obtained bacteria are mixed with protective agents (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) at a ratio of 1:1 according to dry weight, and pre-frozen at -80°C for 24 hours. The bacterial powder was freeze-dried in a vacuum freeze-dryer (PO14416, Telstar LyoQuest-55plus) and pulverized into powder, and then vacuum-packed into an aluminum foil bag.

The number of viable cells of the *Lactiplantibacillus plantarum* JN9 after being fermented for 12 h in the bioreactor was 3.23×10^{9}-5.20×10^{9} CFU/mL. A total of 120-127 g of bacteria were collected by centrifugation of 6L bacterial liquid at 10000rpm and 4 °C for 10 min. The bacteria were mixed with protective agents and freeze-dried to finally obtain 50-60 g of bacteria powder with a viable cell number of 2.81×10^{11}-3.53×10^{11}CFU/g.

Example 4. GABA Production Capacity of the *Lactiplantibacillus Plantarum* JN9

Detection of the *gadB* gene of the *Lactiplantibacillus plantarum* JN9

Fresh bacteria of the *Lactiplantibacillus plantarum* JN9 were collected after culturing at 37°C for 16 h, and DNA was extracted according to the instructions of a DNA extraction kit (9763, Takara, Japan). The amplification primers for glutamate decarboxylase B gene (*gadB* gene) are shown in Table 3. PCR reaction system (25 μL): 2 × Premix Taq™ (R004Q, Takara) 12.5 μL, 1 μL each of upstream and downstream primers (10 μmol/L), 1 μL of template (10 ng), and nuclease-free water to make up to 25 μL. The PCR reaction conditions were denaturation at 94 °C for 5 min, 30 cycles in total of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s,

extension at 72 °C for 1.5 min, and extension at 72 °C for 7 min. PCR products were detected by gel electrophoresis to detect band formation and size. Aspirate 1 μL of the above PCR product, add 5 μL of DNA Loading buffer, mix them and then add the sample to the spotting well, run electrophoresis at 100V for 40 minutes. After the electrophoresis, the bands were observed using a gel imager.

Table 3 Primer sequences (SEQ ID NOs: 4-9)

Gene Name	Upstream primer sequence (5'-3')	Downstream primer sequence (5'-3')
aldh	ATGCTGAAAGAAATGGAAGA	TTAGCGAATCTTAGTTAAGC
adh	ACCGCATACGAGGCATTA	GTACATCCATTCCCAAGCA
gadB	GTAGAATTCATGGCAATGTTATAC GGTAAACAC	CTAGCGGCCGCGTGTGTGAATCCG TATTTCTTAG

Measurement of GABA Production Capacity of the *Lactiplantibacillus plantarum* JN9

After activation, single colonies of the *Lactiplantibacillus plantarum* JN9 (experimental group) and the commercial control bacteria *Lactiplantibacillus plantarum* SG5 (GDMCC 60020) with the ability to produce GABA were picked and inoculated into MRS liquid supplemented with 20 g/L sodium L-glutamate and cultured at 37°C for 48 h. The fresh bacterial liquid was centrifuged at 4°C and 12,000 rpm for 1 minute. 200 μL of the supernatant was taken and placed in a centrifuge tube. 400 μL of 4.2% sodium bicarbonate solution and 200 μL of 1% 2,4-dinitrofluorobenzene solution were added respectively, mixed, and placed in a 60°C water bath for 1 hour. After cooling, 9.2 mL of 0.136% potassium dihydrogen phosphate solution was added, shaken to homogenous, filtered through a 0.22 μm filter membrane and LC-MS was performed. Liquid phase conditions: C18 column, column temperature 35 ℃ ± 5 ℃, gradient elution, mobile phase A of 0.41% anhydrous sodium acetate solution, mobile phase C of acetonitrile. Flow rate is 0.8 mL/min. The detection wavelength is 360nm, and the injection volume is 10 μL. Mass spectrometry conditions: ESI positive ion mode is adopted, and the mass range of mass spectrometry scanning is 20-2000m/z. The ESI source conditions were as follows: capillary voltage 3.5 kV, desolvation temperature 400 ℃, cone voltage 30 V,

desolvation gas flow rate 700 L/h, cone gas flow rate 50 L/h, collision energy 6/20 V.

The results showed that, gene amplification revealed the presence of the *gadB* gene in the genome of the *Lactiplantibacillus plantarum* JN9 ((a) in FIG. 9). LC-MS analysis of the components in the fermentation broth of the *Lactiplantibacillus plantarum* JN9 confirmed that GABA was produced during the fermentation of the *Lactiplantibacillus plantarum* JN9 ((b) in FIG. 9), and the production of GABA increased with extending fermentation time. After 24 hours of fermentation in MRS liquid medium supplemented with 20g/L sodium L-glutamate, the GABA yield for the *Lactiplantibacillus plantarum* JN9 was 10.53 ± 1.70 g/L, and the GABA yield for the commercially available control group *Lactiplantibacillus plantarum* SG5 was 0.42 ± 0.08 g/L. After 48 h of fermentation, the GABA yield of the *Lactiplantibacillus plantarum* JN9 increased to 13.54 ± 0.80 g/L, and the GABA yield of the commercially available control group *Lactiplantibacillus plantarum* SG5 was 0.56 ± 0.03 g/L.

It can be seen that the genome of the *Lactiplantibacillus plantarum* JN9 contains *gadB* gene, and the *Lactiplantibacillus plantarum* has high GABA production capacity and can be used for GABA production. And because it has good safety and alcohol resistance at the same time, it can be used for preparing medicaments for improving sleep and resisting depression, and can also be used for preparing a series of functional (resisting depression, improving sleep, protecting liver, enhancing immunity and/or preventing and treating alcohol hangover) nutraceuticals or food.

Example 5. Alcohol resistance of the *Lactiplantibacillus plantarum* JN9

Acetaldehyde dehydrogenase gene (*aldh*) and alcohol dehydrogenase gene (*adh*) Detection

Fresh bacteria of the *Lactiplantibacillus plantarum* JN9 were collected after culturing at 37°C for 16 h, and DNA was extracted according to the instructions of a DNA extraction kit (9763, Takara, Japan). The amplification primers for *aldh* gene and *adh* gene are shown in Table 3. PCR reaction system (25 μL): 2 × Premix Taq ™ (R004Q, Takara) 12.5 μL, 1 μL each of upstream and downstream primers (10 μmol/L), 1 μL of template (10 ng), and nuclease-free water to make up to 25 μL. The PCR reaction conditions were denaturation at 98 °C for 4 min,

32 cycles in total of denaturation at 98 °C for 10 s, annealing at 56 °C for 15 s, extension at 72 °C for 30 s, and extension at 72 °C for 7 min. PCR products were detected by electrophoresis to detect band formation and size. Aspirate 1 μL of the above PCR product, add 5 μL of DNA Loading buffer, mix them and add the sample to the spotting well, and run electrophoresis at 100V for 40 minutes. After the electrophoresis, the bands were observed using a gel imager.

Evaluation of the resistance of the *Lactiplantibacillus plantarum* JN9 to different concentrations of alcohol

After activation, the *Lactiplantibacillus plantarum* JN9 was inoculated into MRS broth and cultured at 37°C for 16 h. The bacterial liquid was then adjusted to 10^8 CFU/mL. The bacterial suspension was added to MRS liquid medium containing anhydrous ethanol at a ratio of 1:1000 to final ethanol concentrations of 3%, 7%, 12%, and 15%. The culture was incubated at 37°C for 24 hours. Strain growth was measured by viable count.

Detection of Acetaldehyde Dehydrogenase Activity of the *Lactiplantibacillus Plantarum* JN9

The *Lactiplantibacillus plantarum* JN9 (experimental group), *Lactobacillus rhamnosus* LGG (control 1) and commercially available *Lactiplantibacillus plantarum* YLA1 (CCTCC NO: M2020289, control 2) with acetaldehyde dehydrogenase activity were activated and then inoculated into MRS broth respectively. After static culture at 37°C for 16 h, the bacteria were collected by centrifugation, washed twice with sterile PBS buffer and resuspended, and the OD was adjusted to 1. The sample to be tested was obtained by ultrasonic disruption in ice bath (power 300w, ultrasonic 5s, interval 7s, total time 15min), and PBS was used as blank control. According to Table 4, the enzyme activity reaction systems of the *Lactiplantibacillus plantarum* JN9, LGG, YLA1 and blank samples were respectively configured and incubated at 37 °C for 30 minutes. Record the absorbance values A1 and A2 at 340 nm before and after incubation of the sample, and calculate ΔA assay tube = A2 assay tube - A1 assay tube, ΔA blank tube = A2 blank tube - A1 blank tube, and ΔA = ΔA assay tube - ΔA blank tube. Definition of enzyme activity: one enzyme activity unit is defined as that produce 1 μmol of

NADH per milliliter of sample per minute.

ALDH enzyme activity (U/mL) = $\Delta A \div (\varepsilon \times d) \times 10^6 \times$ V reaction total ÷ V sample ÷ T

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well plate optical path, 0.6 cm; V reaction total: total volume of the reaction system; V sample: sample volume in the reaction system; T: reaction time, 30 min; 10^6 unit conversion factor, 1 mol = 10^6 μmol

Table 4 Enzyme activity assay reaction system

Reagent	Volume
Sample	40 μ L
100mmol/L acetaldehyde	20 μ L
20mmol/L NAD	30 μ L
1 mmol/L β-mercaptoethanol	3 μ L
1 mmol/L Tris-HCl	30 μ L
ddH_2O	77 μ L
Total Volume	200 μ L

By amplifying the *adh* gene and *aldh* gene of the *Lactiplantibacillus plantarum* JN9, the electrophoresis results of PCR amplification products are shown in FIG. 10. Both gene bands are single, with sizes of about 500 bp and 1400 bp, respectively, which were consistent with the expected sizes. It shows that the genome of the *Lactiplantibacillus plantarum* JN9 contains *aldh* and *adh*. Through alcohol resistance experiment, it was concluded that the *Lactiplantibacillus plantarum* JN9 had good growth ability in MRS medium containing 3%, 7% and 12% ethanol, and could tolerate MRS medium containing 15% ethanol. After 16 hours of incubation, the live bacteria test found that the survival rate of the bacteria was 16.67%. The acetaldehyde dehydrogenase activity assay revealed that the acetaldehyde dehydrogenase activity of *Lactiplantibacillus plantarum* JN9 strain was 5.29 (μmol/mL), the commercial control group *Lactiplantibacillus plantarum* YLA (control 2) contains *adh* gene and *aldh* gene and its acetaldehyde dehydrogenase activity was 3.22 (μmol/mL), and *Lactobacillus rhamnosus* LGG (Control 1) was lack of the *aldh* gene and showed no detectable acetaldehyde dehydrogenase activity.

It can be seen that the genome of the *Lactiplantibacillus plantarum* JN9 strain contains acetaldehyde dehydrogenase gene (*aldh*) and alcohol dehydrogenase gene (*adh*). The strain has

high acetaldehyde dehydrogenase activity and strong alcohol resistance, and can be used to prepare medicament for protecting liver and/or preventing and treating alcohol hangover.

Embodiments described above are used for illustration of this application but not to limit the scope of this application, and the scope of this application is determined by the appended claims.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavor to which this specification relates.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

CLAIMS

1. A *Lactiplantibacillus plantarum* JN9, wherein the *Lactiplantibacillus plantarum* has a taxonomic designation of *Lactiplantibacillus plantarum* and a Depositary Accession Number of CCTCC NO: M 20241647.

2. A formulation prepared from the *Lactiplantibacillus plantarum* JN9 of claim 1, wherein the formulation is selected from one or both of the following:

1) a bacterial dry product produced from the *Lactiplantibacillus plantarum* JN9; and

2) a bacterial liquid product produced from the *Lactiplantibacillus plantarum* JN9.

3. A probiotic composition comprising the *Lactiplantibacillus plantarum* JN9 of claim 1 or the formulation of claim 2.

4. The probiotic composition of claim 3, wherein 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.

5. Use of the *Lactiplantibacillus plantarum* JN9 of claim 1 in producing GABA.

6. Use of the *Lactiplantibacillus plantarum* JN9 of claim 1 in preparation of a medicament for preventing and treating alcohol hangover.

2025223817 28 Aug 2025

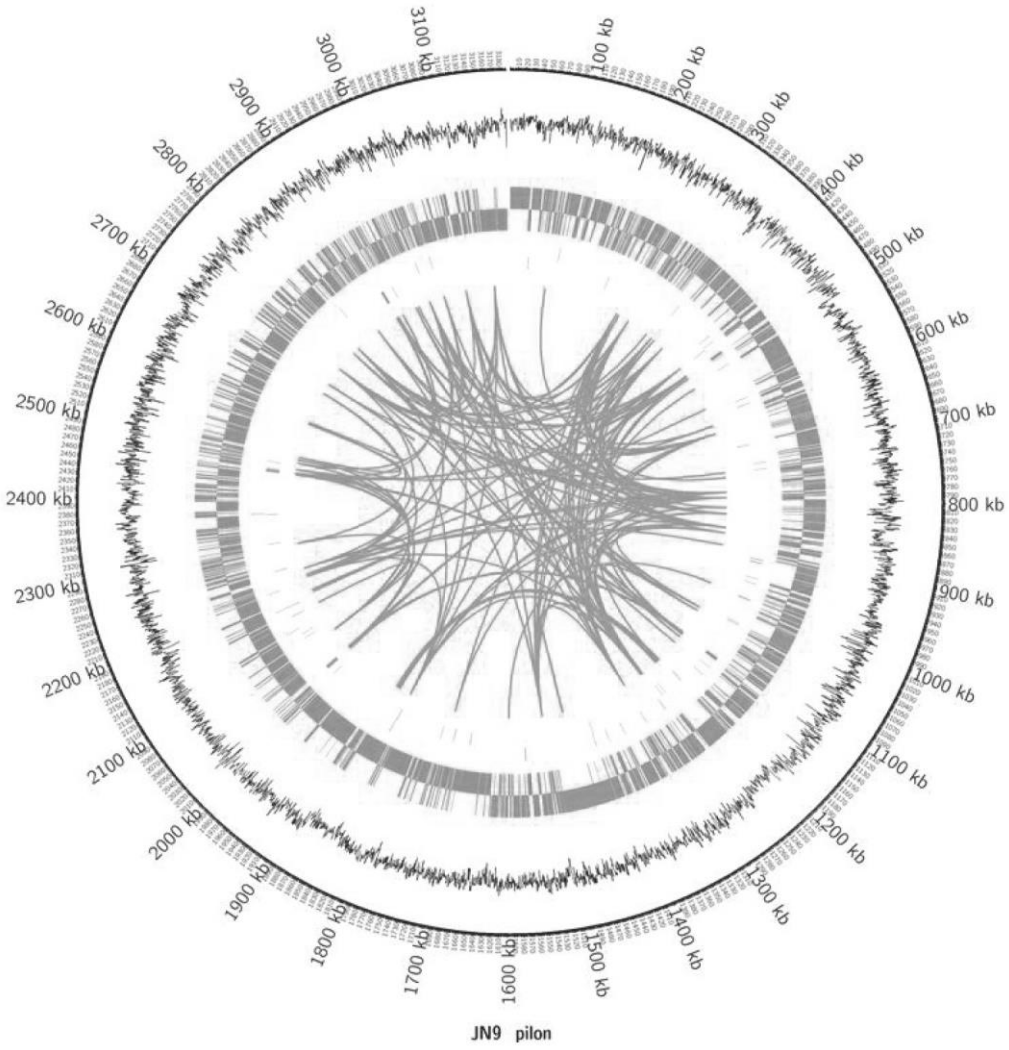

FIG. 1

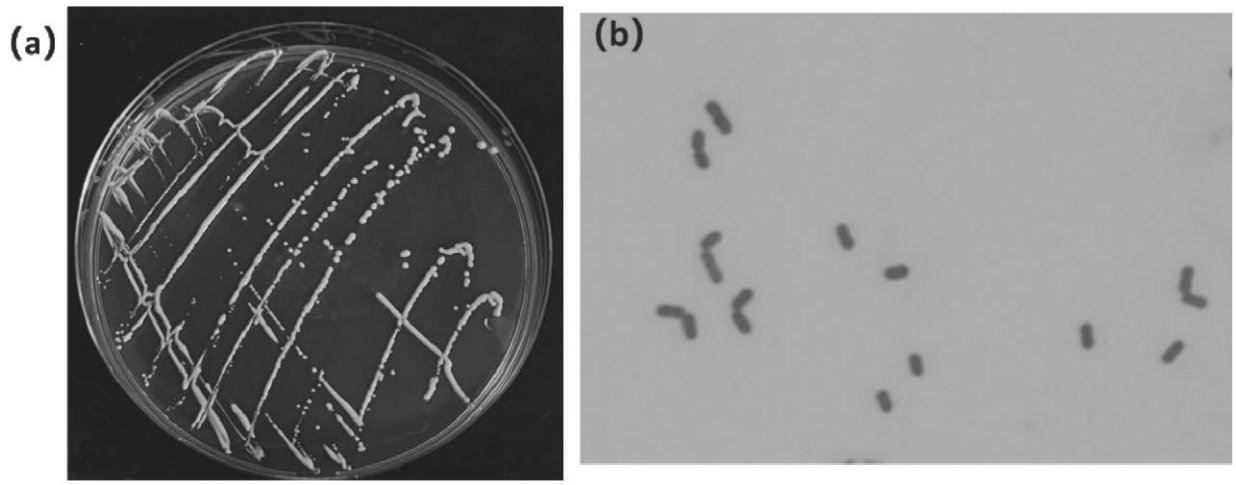

FIG. 2

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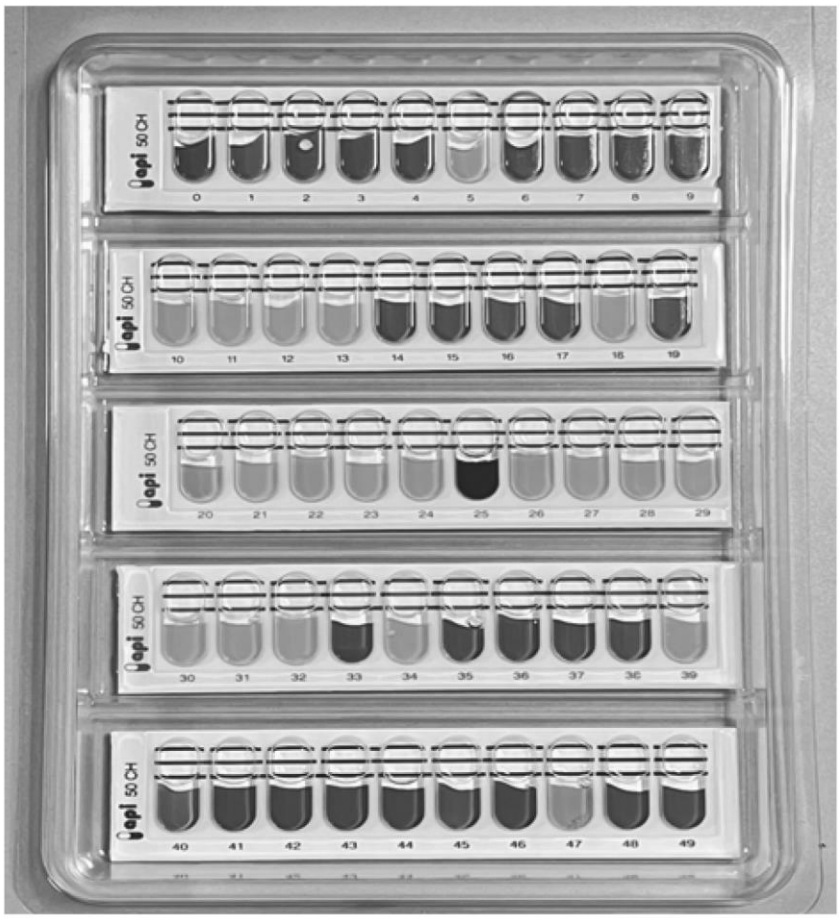

FIG. 3

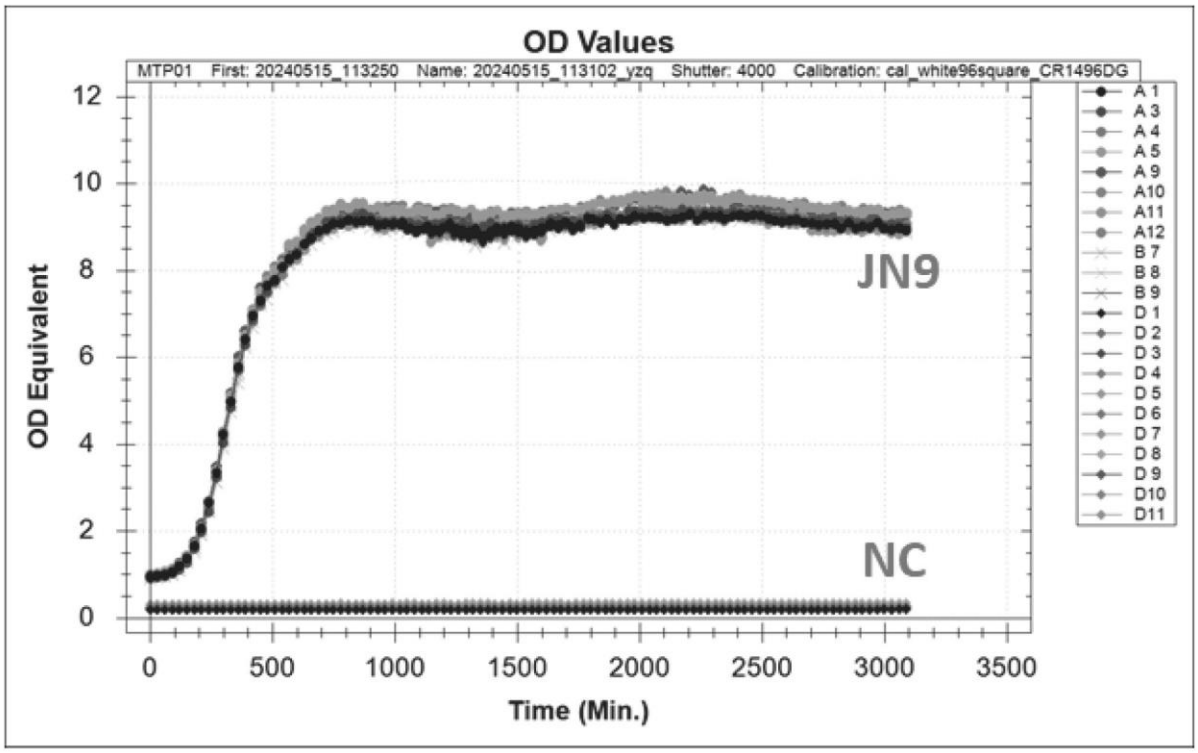

FIG. 4

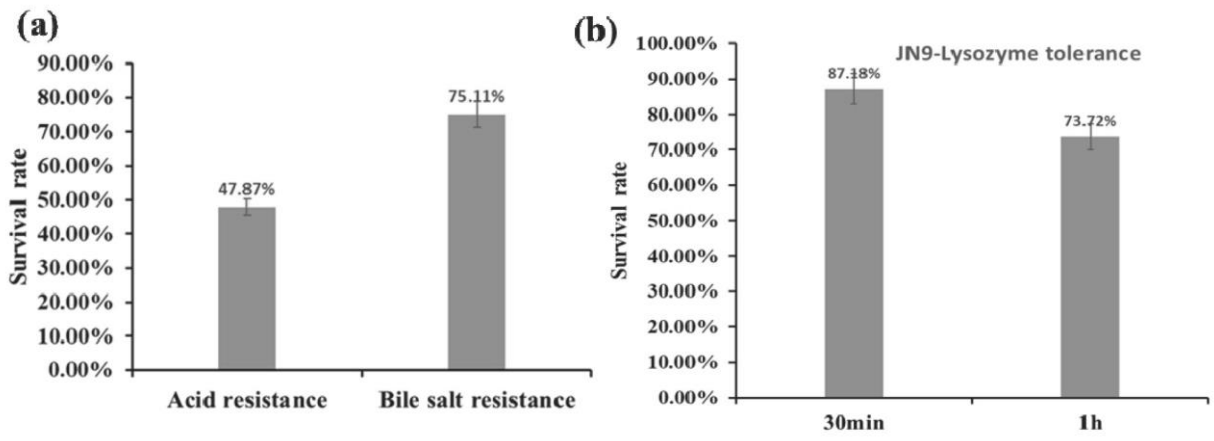

FIG. 5

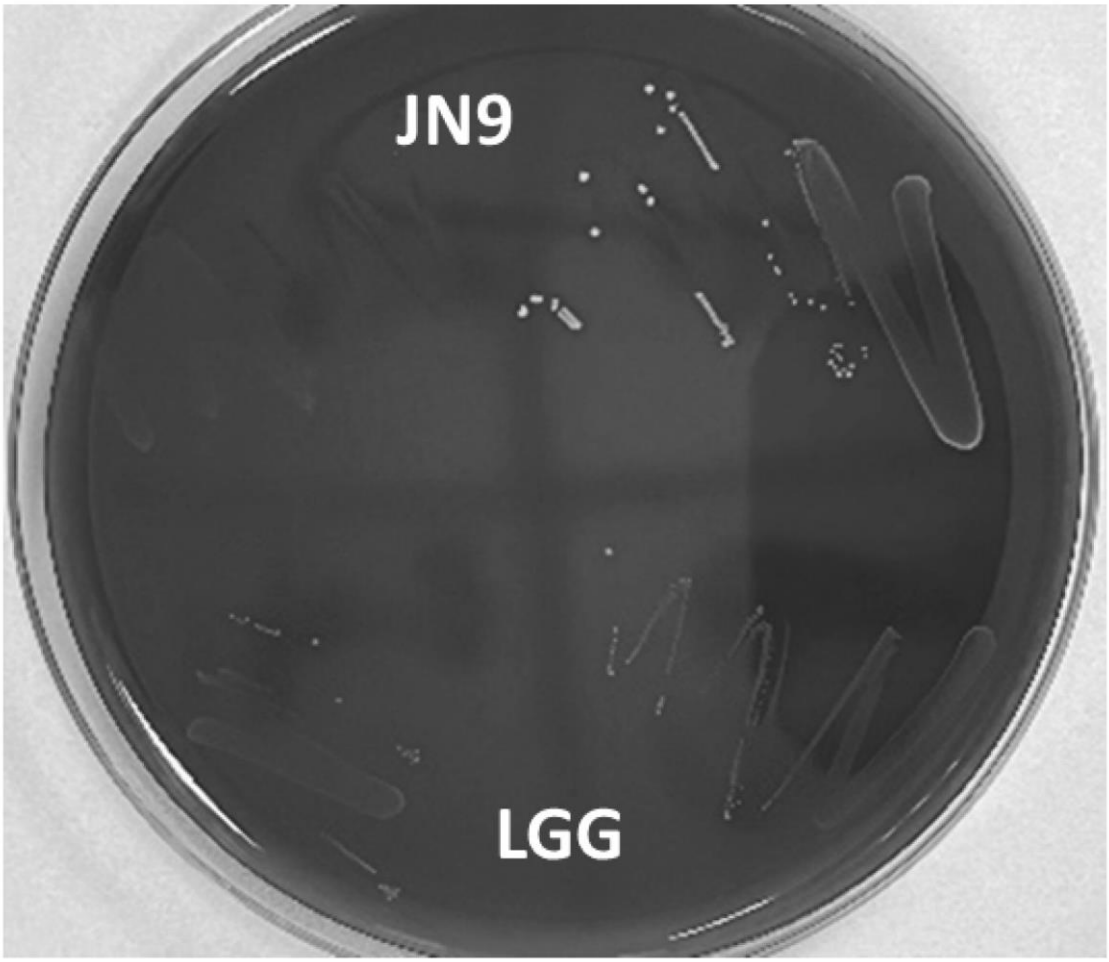

FIG. 6

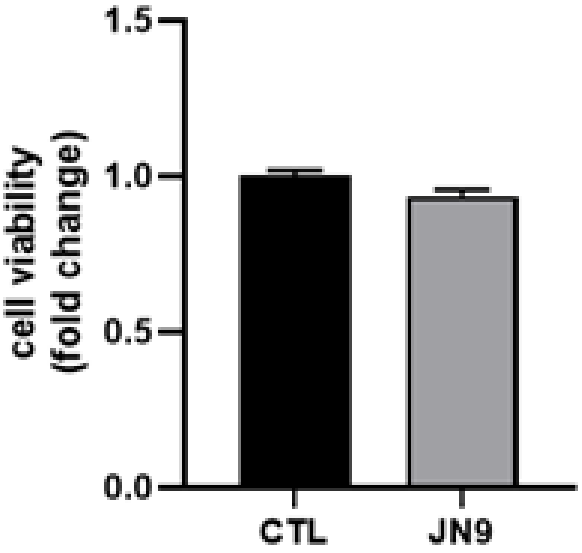

FIG. 7

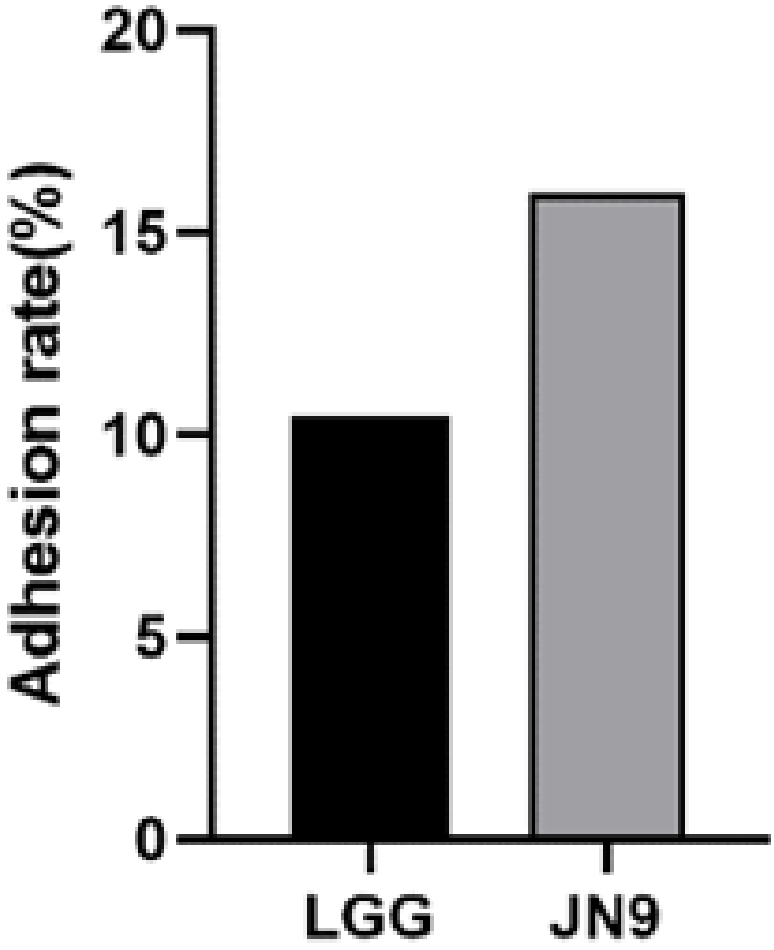

FIG. 8

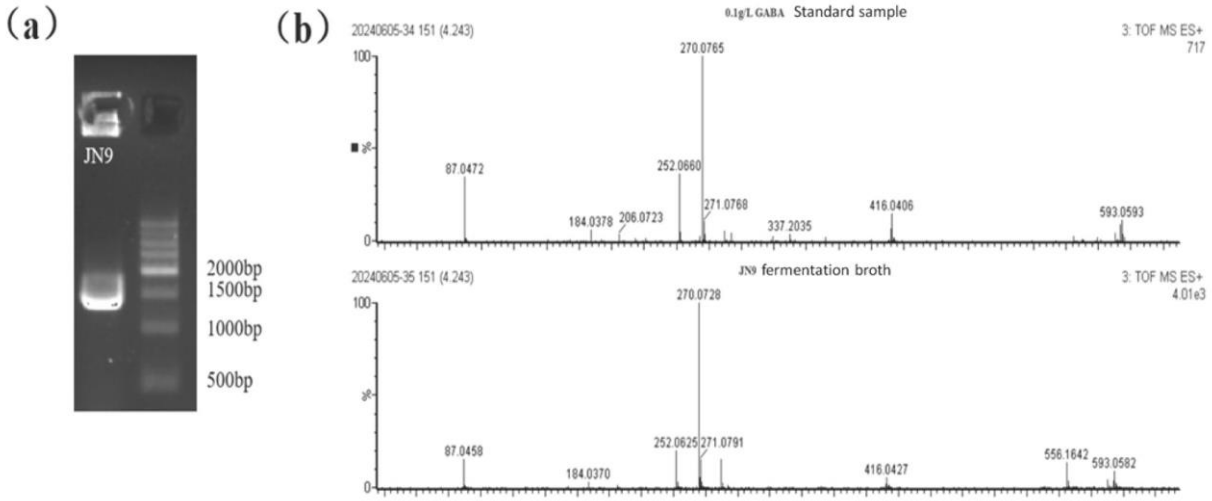

FIG. 9

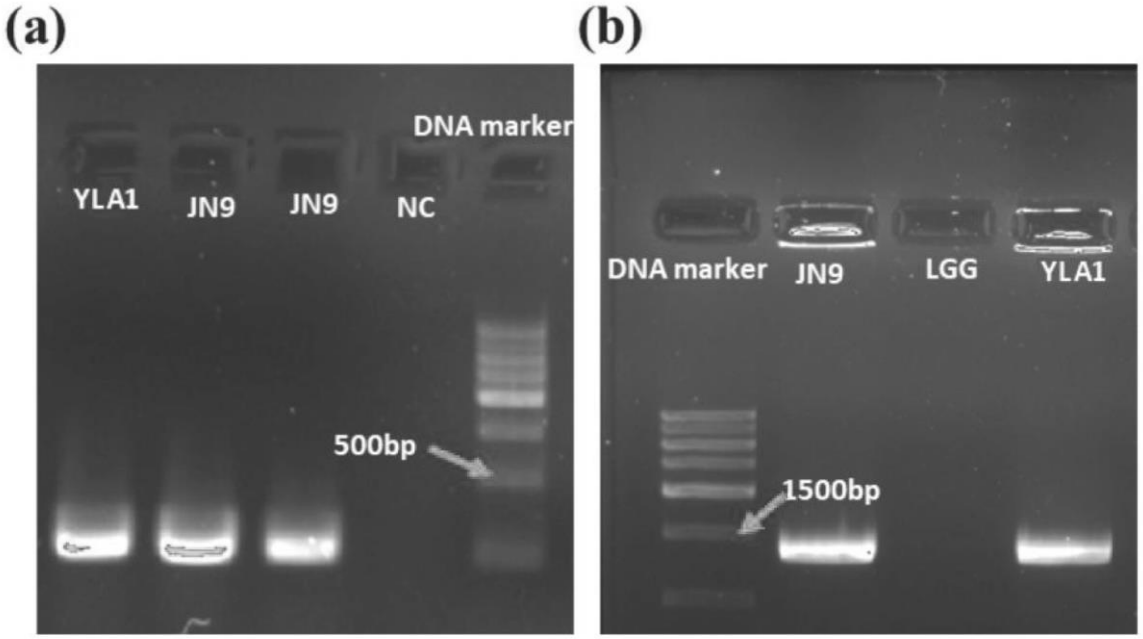

FIG. 10

Sequence Listing

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1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.3.0
1-5	Production Date	2024-08-29
1-6	Original free text language code	
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	092413257-PZ
2-5	Earliest priority application: IP Office	
2-6	Earliest priority application: Application number	
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2-8en	Applicant name	
2-8	Applicant name: Name Latin	ADM (Shanghai) Management Co., Ltd.
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	
2-11	Sequence Total Quantity	9

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3-2-2	Molecule Type	RNA
3-2-3	Length	19
3-2-4	Features	**source 1..19**
	Location/Qualifiers	mol_type=other RNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-2-5	Residues	ggttaccttg ttacgactt 19
3-3	**Sequences**	
3-3-1	Sequence Number [ID]	3
3-3-2	Molecule Type	RNA
3-3-3	Length	1443
3-3-4	Features	**source 1..1443**
	Location/Qualifiers	mol_type=rRNA
		organism=Lactiplantibacillus plantarum
	NonEnglishQualifier Value	
3-3-5	Residues	cttagcggct ggttcctaaa aggttacccc accgactttg ggtgttacaa actctcatgg 60 tgtgacgggc ggtgtgtaca aggcccggga acgtattcac cgcggcatgc tgatccgcga 120 ttactagcga ttccgacttc atgtaggcga gttgcagcct acaatccgaa ctgagaatgg 180 ctttaagaga ttagcttact ctcgcgagtt cgcaactcgt tgtaccatcc attgtagcac 240 gtgtgtagcc caggtcataa ggggcatgat gatttgacgt catccccacc ttcctccggt 300 ttgtcaccgg cagtctcacc agagtgccca acttaatgct ggcaactgat aataagggtt 360 gcgctcgttg cgggacttaa cccaacatct cacgacacga gctgacgaca accatgcacc 420 acctgtatcc atgtccccga agggaacgtc taatctctta gatttgcata gtatgtcaag 480 acctggtaag gttcttcgcg tagcttcgaa ttaaaccaca tgctccaccg cttgtgcggg 540 cccccgtcaa ttcctttgag tttcagcctt gcggccgtac tccccaggcg gaatgcttaa 600 tgcgttagct gcagcactga agggcggaaa ccctccaaca cttagcattc atcgtttacg 660 gtatggacta ccagggtatc taatcctgtt tgctacccat actttcgagc ctcagcgtca 720 gttacagacc agacagccgc cttcgccact ggtgttcttc catatatcta cgcatttcac 780 cgctacacat ggagttccac tgtcctcttc tgcactcaag tttcccagtt tccgatgcac 840 ttcttcggtt gagccgaagg ctttcacatc agacttaaaa aaccgcctgc gctcgcttta 900 cgcccaataa atccggacaa cgcttgccac ctacgtatta ccgcggctgc tggcacgtag 960 ttagccgtgg ctttctggtt aaataccgtc aatacctgaa cagttactct cagatatgtt 1020 cttctttaac aacagagttt tacgagccga aacccttctt cactcacgcg gcgttgctcc 1080 atcagacttt cgtccattgt ggaagattcc ctactgctgc ctcccgtagg agtttgggcc 1140 gtgtctcagt cccaatgtgg ccgattaccc tctcaggtcg gctacgtatc attgccatgg 1200 tgagccgtta ccccaccatc tagctaatac gccgcgggac catccaaaag tgatagccga 1260 agccatcttt caaactcgga ccatgcggtc caagttgtta tgcggtatta gcatctgttt 1320 ccaggtgtta tcccccgctt ctgggcaggt ttcccacgtg ttactcacca gttcgccact 1380 cactcaaatg taaatcatga tgcaagcacc aatcaatacc agagttcgtt cgactgcatg 1440 tat 1443
3-4	**Sequences**	
3-4-1	Sequence Number [ID]	4
3-4-2	Molecule Type	RNA
3-4-3	Length	20
3-4-4	Features	**source 1..20**
	Location/Qualifiers	mol_type=other RNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-4-5	Residues	atgctgaaag aaatggaaga 20
3-5	**Sequences**	
3-5-1	Sequence Number [ID]	5
3-5-2	Molecule Type	RNA
3-5-3	Length	20
3-5-4	Features	**source 1..20**
	Location/Qualifiers	mol_type=other RNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-5-5	Residues	ttagcgaatc ttagttaagc 20
3-6	**Sequences**	

3-6-1	Sequence Number [ID]	6	
3-6-2	Molecule Type	RNA	
3-6-3	Length	18	
3-6-4	Features	**source 1..18**	
	Location/Qualifiers	mol_type=other RNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-6-5	Residues	accgcatacg aggcatta	18
3-7	**Sequences**		
3-7-1	Sequence Number [ID]	7	
3-7-2	Molecule Type	RNA	
3-7-3	Length	19	
3-7-4	Features	**source 1..19**	
	Location/Qualifiers	mol_type=other RNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-7-5	Residues	gtacatccat tcccaagca	19
3-8	**Sequences**		
3-8-1	Sequence Number [ID]	8	
3-8-2	Molecule Type	RNA	
3-8-3	Length	33	
3-8-4	Features	**source 1..33**	
	Location/Qualifiers	mol_type=other RNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-8-5	Residues	gtagaattca tggcaatgtt atacggtaaa cac	33
3-9	**Sequences**		
3-9-1	Sequence Number [ID]	9	
3-9-2	Molecule Type	RNA	
3-9-3	Length	34	
3-9-4	Features	**source 1..34**	
	Location/Qualifiers	mol_type=other RNA	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-9-5	Residues	ctagcggccg cgtgtgtgaa tccgtatttc ttag	34