



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Genomic and safety assessment of *Niallia circulans* SWUN-Y10 reveals probiotic potential

Yangyang Yang¹, Haijun Xu¹, Yuanyuan Xi¹, Yanting Guo¹, Yuan Liu², Likou Zou³, Chenglin, Zhu¹, Daoying, Wang⁴, Junni Tang^{1*}

¹ College of Pharmacy and Food, Southwest Minzu University, Chengdu 610041, Sichuan, P. R. China.

² College of Grassland Resources, Southwest Minzu University, Chengdu 610225, Sichuan, P. R. China.

³ College of Resources, Sichuan Agricultural University, Chengdu, 611130, Sichuan, P. R. China.

⁴ Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, P. R. China.

ABSTRACT: This study assessed the safety and probiotic properties of *Niallia circulans* SWUN-Y10, through both in vitro methods such as antibiotic susceptibility testing, biogenic amine testing, and virulence gene analysis, and in vivo methods including biochemical testing, routine blood index testing, bacterial displacement capacity, and histopathology analysis. The whole genome sequence of SWUN-Y10 was determined to be 5074781 bp in length, with an average G+C content of 35.41%, which encoded totally 5269 genes. Genomic analysis confirmed the absence of genes encoding critical virulence factors and identified only a single chromosomal antibiotic resistance gene (*vmIR*); alongside genes associated with carbohydrate metabolism and stress tolerance were also identified. In vitro phenotypic characteristics demonstrated the strain was also safety, evidenced by non-hemolytic activity, lack of biogenic amine production, and broad antibiotic susceptibility. Furthermore, SWUN-Y10 exhibited robust in vitro probiotic attributes, including high tolerance to simulated gastrointestinal stress (pH 2.0, 0.3% bile salts), significant auto-aggregation, and substantial cell surface hydrophobicity. The results of 14-day acute oral toxicity test showed that *Niallia circulans* SWUN-Y10 (10^{11} CFU/kg body weight) did not cause significant effect on the normal growth, erythrocytes, white blood cells, and other routine blood parameters in mice. Furthermore, there was no bacteria migration observed in the liver, kidney, spleen, or heart during the feeding trial, which indicated that *Niallia circulans* SWUN-Y10 did not cause any infections in vivo. Moreover, H&E staining showed no evidence of lesion damage to the liver. To sum up, *Niallia circulans* SWUN-Y10 exhibits a promising safety and functional probiotic properties, supporting its potential use in future as a starter culture for food fermentation or as a probiotic supplement.

Keywords: *Niallia circulans*; genome sequencing; in vitro and in vivo; safety evaluation; probiotic properties

1. Introduction

Probiotics are defined as live microorganisms that exert beneficial effects on the host following ingestion in sufficient quantities (Bhatia et al., 2025). The extensive historical application of probiotic strains, including species from *Bifidobacterium*, *Lactobacillus*, and *Bacillus*, in traditional food processes forms the foundation for their current utilization in advanced health-food and biotechnological industries (Sibanda et al., 2024;

*Corresponding author
junneytang@swun.edu.cn

Received 30 October 2025
Received in revised form 10 December 2025
Accepted 24 December 2025

Vijayaganapathi & Mohanasrinivasan, 2025a). The persistent demand for enhanced probiotic properties sustains the ongoing exploration of new strains (Vijayaganapathi & Mohanasrinivasan, 2025b). As such, spore-forming *Bacillus* species represent promising probiotic alternatives, as their spores are dormant, resilient structures that enable easy storage, long-term preservation, and simplified production processes. The inherent resistance of spores to external contaminants significantly enhances the purity and operational stability of fermentation agents (Freire et al., 2024). Owing to their spore formation, *Bacillus* strains exhibit enhanced resistance to low pH, resulting in superior shelf-life and gastrointestinal survival compared to non-spore-forming probiotics, which translates to greater effectiveness in the food and animal feed industries (Feng et al., 2025). Numerous *Bacillus* species, such as *Bacillus subtilis* and *Bacillus velezensis*, have documented safety and functional profiles suitable for use as oral probiotics and dietary supplements (Cui et al., 2024; Soni et al., 2022). However, there has not been sufficient study on the probiotic characteristics and safety assessment of *Niallia circulans*.

According to World Health Organization (WHO), probiotics are “live microorganisms” that, when consumed in sufficient quantities, provide health benefits to the host (Liaqat et al., 2025). Therefore, a critical prerequisite for probiotics is a demonstrated safety profile, encompassing GRAS/QPS regulatory status and a minimal risk of antibiotic resistance gene transfer (Duche et al., 2023; Koutsoumanis et al., 2020). Furthermore, critical for probiotics is the ability to withstand processing and gastrointestinal transit; strains with acid and bile tolerance, coupled with epithelial adhesion, demonstrate superior survival (Mendonça et al., 2022).

The genus *Niallia*, created by the reclassification of *Bacillus circulans* to *Niallia circulans* (Patel & Gupta, 2020), is one of the least characterized genera within the *Bacillaceae*. *N. circulans* is a Gram-positive, spore-forming, rod-shaped species. It is usually found in soil and the non-pathogenic *N. circulans* and other *Bacillus* spp. are used for the industrial production of enzymes, especially proteinases (Hossain et al., 2023). As a potential probiotic, there is currently a lack of research evidence on *N. circulans*. Hence, in this study, our aim is to evaluate the safety of *N. circulans* SWUN-Y10 in vitro and in vivo. The virulence factors, and antibiotic-resistance genes in genomes of *N. circulans* SWUN-Y10 were predicted. The assessment of hemolytic activity, antibiotic resistance, and biogenic amine production was conducted. The abilities related to the acid resistance, salt tolerance, cell surface hydrophobicity and self-aggregation properties of the SWUN-Y10 probiotic were evaluated. Furthermore, an acute oral toxicity study was performed over 14 days to evaluate the safety of the strain in mice, with assessments including body weight, food intake, organ indices, hematological and serum biochemical profiles, and bacterial translocation. Our results confirm that *N. circulans* SWUN-Y10 exhibits a promising safety and functional probiotic properties.

2. Materials and methods

2.1 Materials

N. circulans SWUN-Y10 used in this study was screened by our laboratory and has been preserved in the China General Microbiological Culture Collection Center (CGMCC, No. 31985). The complete genomic

sequence of *N. circulans* SWUN-Y10 is available in the GenBank database, registered under accession numbers CP185931-CP185932. *N. circulans* SWUN-Y10 was cultivated in an TSB broth medium (Hopebio, Qingdao, China) and cultured at 37 °C for 24 h. Bacteria for in vivo test was cultivated three times. To achieve desired count of bacterial species (1×10^{10} CFU/mL), cells were harvested by centrifugation at $9000 \times g$ for 10 min followed by washing with sterile saline and finally suspended in sterile saline. Animal trials were approved by the Ethics Committee of Southwest Minzu University (SMU-202401149). SPF-grade Balb/c strain female and male mice, aged six weeks, were purchased from Chengdu Dashuo experimental animal Co., Ltd. (Chengdu, China) and housed in an SPF-grade laboratory animal facility under a standard 12h:12h light: dark cycle with free access to water and food. Prior to experimentation, mice were allowed 7 days to acclimatize to the housing conditions.

2.2 Whole-genome sequencing

Genomic DNA was extracted from *N. circulans* SWUN-Y10 using a bacterial genomic DNA extraction kit (Tiangen, Beijing, China). The integrity, concentration, and purity of the extracted DNA were subsequently assessed. *N. circulans* SWUN-Y10 genome was sequenced using the Illumina platform and Nanopore platform by Beijing Qingke Biotechnology Co., LTD. For genome assembly, the filtered reads were assembled by Canu v1.5 software, and then circlator v1.5.5 was taken to cyclizing assembly genome. For functional annotation, the predicted protein was blast against KEGG, eggNOG, and further used for GO annotation (Li et al., 2019; Liang et al., 2024a; Lodi et al., 2025). CAZy database was used to annotate carbohydrate-associated enzymes.

2.3 Antibiotic susceptibility

The antibiotic susceptibility of *N. circulans* SWUN-Y10 was assessed through the disk diffusion method recommended by the Clinical and Laboratory Standard Institute (CLSI) (Lu et al., 2023). Sixteen types of antibiotic discs (Hangzhou Binhe Microorganism Reagent Co., Ltd., Hangzhou, China) were selected for the bacterial drug sensitivity test. The concentration of *N. circulans* SWUN-Y10 liquid was adjusted to 1×10^8 CFU/mL. Then, 100 μ L of bacterial suspension was spread on Tryptic Soy Agar (TSA) plates, and antibiotic discs were aseptically placed onto the surface once dry. After an incubation period of 5 min, the plates were incubated for 24 h at 37 °C. The diameter of the inhibition zone was measured for each antibiotic plate and the sensitivities of the drugs were evaluated in accordance with the standard procedure for microbiological susceptibility testing (Liang et al., 2024a; Zheng et al., 2024).

2.4 Hemolysis activity

The SWUN-Y10 strain was cultured for two consecutive generations, inoculated onto Columbia blood agar plates containing 5% sheep blood, and incubated at 37 °C for 48 h to assess the formation of a hemolytic ring. *Staphylococcus aureus*, characterized by its distinct β -hemolytic activity, was employed as a positive control to aid in the accurate interpretation of the hemolytic patterns observed in SWUN-Y10 (Sabino et al., 2024). Uninoculated TSB medium was served as a negative control.

2.5 Amino acid decarboxylase test

1% (v/v) bacterial suspension was inoculated into biochemical identification tubes containing various amino acids (lysine, arginine, and ornithine) and amino acid decarboxylase controls (Hopebio, Qingdao, China). Liquid paraffin was added to isolate the air, and the tubes were incubated at 37 °C for 48 h. Color changes in the medium were observed visually. *Escherichia coli* ATCC25922 was the positive control and *Shigella flexneri* CICC 11868 was negative control (Wang et al., 2024).

2.6 Assessment of probiotic properties

2.6.1 Acid and bile tolerance of *N. circulans* SWUN-Y10

A bacterial suspension was prepared by resuspending the cultured test organisms in phosphate-buffered saline (PBS) at a concentration of 1×10^9 CFU/mL. A 2% inoculum was transferred into trypticase soy broth (TSB) medium, which were adjusted to pH 2, 3, 4, and 5 with hydrochloric acid. All experiments were performed in triplicate for each pH condition. After 2 h incubation at 37 °C, the OD₆₀₀ was measured, and the bacterial survival rate was calculated (Survival rate = OD₆₀₀ value of treated medium/OD₆₀₀ value of untreated medium $\times$ 100%) (Liang et al., 2024a).

The cultured bacteria were used to inoculate standard nutrient media at a final concentration of 1%. Pig bile salt concentrations of 0.1, 0.2, and 0.3 g/100 mL were tested in the media, each with three independent replicates. Following incubation at 37 °C for 2 h, the OD₆₀₀ was measured (Survival rate = OD₆₀₀ value of treated medium/OD₆₀₀ value of untreated medium $\times$ 100%) (Xu et al., 2025).

2.6.2 Cell surface hydrophobicity of *N. circulans* SWUN-Y10

The cell surface hydrophobicity of the *N. circulans* SWUN-Y10 strain was determined following the protocol of Dabadé et al. (Dabadé et al., 2021). Following a 24-hour cultivation at 37 °C, bacterial cells were harvested by centrifugation, washed twice with PBS, and subsequently resuspended in 2 mL of PBS to an OD₆₀₀ of 0.5 (A_0). An equal volume of various solvents (xylene, chloroform or ethyl acetate) was added to each suspension. The mixture was then vortexed for 2 min and incubated at 37 °C for 40 min. The optical density at 600 nm (OD₆₀₀) of the aqueous phase was recorded (denoted as A_1). The bacterial adhesion to solvent (BAS) percentage was then calculated using the following formula: $[(A_0 - A_1) / A_0] \times 100\%$.

2.6.3 Auto-aggregation analysis of *N. circulans* SWUN-Y10

The protocol of Li et al. (2020) was employed to assess the auto-aggregation capacity of *N. circulans* SWUN-Y10. Cell suspensions were standardized to an OD₆₀₀ of 1.0, aliquoted into test tubes, and vortexed for 30 s. Following this, the tubes were transferred to a 37 °C incubator. The supernatant was collected at specified time points (0, 2, 4, 6, 10, and 24 h), and its absorbance at 600 nm was determined. The autoaggregation rates were determined as follows: $[(A_0 - A_1) / A_0] \times 100$ (where A_0 denotes the absorbance at time (t) = 0, A_1 denotes the absorbance at t = 2, 4, 6, 10, or 24 h).

2.7 Acute oral toxicity test

The acute oral toxicity test was performed in compliance with the limit test standards specified in the OECD Guideline No. 425 (OECD, 2022) and U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations (GLP). Twelve healthy mice were randomly divided into two groups consisting of 6 females. Among these, the initial doses were 1×10^{11} CFU/kg body weight. The dose for the subsequent animal was determined based on the short-term outcomes of the administered dose. Based on the safety findings from the short-term study, all the mice of experimental group were given at a dose of 1×10^{11} CFU/kg body weight. The mice of control group were given equal amounts of saline. The bacterial suspension was administered to the mice via oral gavage using a sterile pipette. Following the short-term evaluation, surviving animals were continuously monitored over an extended period of 14 days. Subsequently, they were euthanized by decapitation on day 15, following an overnight fast. A comprehensive necropsy was performed on each animal as part of the assessment protocol.

2.8 Body weight, feed intake and organ index

The body weight of each mouse was measured every day in both the control and experimental groups. The average weight within each group was subsequently compared. The general behavior and feed intake of all the mice were recorded daily. At the end of the experiment, the experimental animals were euthanized, following which selected visceral organs (heart, liver, spleen, lung and kidney) were surgically excised. Organ weights were precisely measured using analytical balances to determine relative organ weight indices, calculated as the ratio of organ mass (g) to total body weight (g) of the corresponding animal (Cheng et al., 2022; Samtiya et al., 2020).

2.9 Hematological and serum biochemical evaluation

Prior to the end of the test, mice were fasted for 12 h, after which blood samples were collected via retro-orbital puncture, with a volume of 0.8~1.0 mL obtained each mouse. The blood samples were then divided into two equal portions. One portion was collected into EDTA-coated tubes for routine hematological analysis. Routine blood parameters were measured using a fully automated hematology analyzer (BC-2800Vet, Mindray, China): white blood cells (WBC), lymphocytes (Lymph), monocytes (Mon), granulocytes (Gran), lymphocyte percentage (Lymph%), monocyte percentage (Mon%), granulocyte percentage (Gran%), red blood cells (RBC), hemoglobin concentration (HGB), hematocrit value (HCT), mean corpuscular value (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and platelet crit (PCT) were measured.

The other portion of blood samples for serum biochemical evaluation was placed in 1.5 ml centrifuge tubes, which were left to stand for one hour without the addition of EDTA, and then centrifuged at 3000 rpm for 10 min to separate the upper serum layer for subsequent analysis. Serum biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), urea (UREA), glucose (GLU), total cholesterol (TC), and triglycerides (TG) were quantified using a BS-200E clinical chemistry analyzer (Mindray Medical International, Shenzhen, China) following standardized

operating protocols (Fan et al., 2023). Analytical validation was performed in accordance with manufacturer specifications and peer-reviewed methodologies to ensure assay reliability.

2.10 Evaluation of *N. circulans* SWUN-Y10 translocation capacity

To determine whether *N. circulans* SWUN-Y10 could translocate from the gastrointestinal tract to other organs, a targeted experiment was performed. Upon completion of the feeding period, mice were euthanized and dissected to assess the gross morphology (color and size) of the heart, liver, spleen, and kidneys. The organs were collected using sterile scissors and placed into sterile saline solution at a concentration of 1 g/mL. The samples were then homogenized and disrupted. A 100 μ L aliquot of the tissue homogenate was aseptically inoculated onto TSA and cultured at 37 °C for 48 h (Gu et al., 2024).

2.11 Histopathology

A comprehensive gross pathological examination was performed on the liver. Tissue samples were collected from live subjects, embedded in paraffin and sectioned at a thickness of 4 μ m in the coronal plane. Following this, the paraffin-embedded sections from the mice were dewaxed, rehydrated, and subsequently stained with hematoxylin and eosin (H&E). The sections were dehydrated and sealed prior to examination under a light microscope to assess the histopathological features. Images were captured for further analysis using BA210 Digital triocular camera microscope (Macaudy Industrial Group Co., LTD) (Mačianskienė et al., 2023).

2.12 Statistical analysis

Data analysis was performed using SPSS 26.0, with results presented as the mean $\pm$ standard deviation (SD). Inter-group differences were assessed using one-way ANOVA, and Tukey's multiple range test was carried out for significant analysis. A *P*-value < 0.05 was considered statistically significant. Graphs were generated using GraphPad Prism 9.5.

3. Results and discussion

3.1 Basic genome information of *N. circulans* SWUN-Y10

The genome of *N. circulans* SWUN-Y10 consists of one circular chromosome and one linear plasmids. The whole genome sequence is 5074781 bp in length, with an average G+C content of 35.41% (detailed in Table 1). The plasmad size is 401667 bp. Prediction showed the genome of *N. circulans* SWUN-Y10 encoded totally 5269 genes, and encoded 83 tRNAs and 33 rRNAs. Moreover, other gene components were predicted, including 18765 repeated sequences, 18 gene islands, 7 prophages and 67 potential CRISPR sequences.

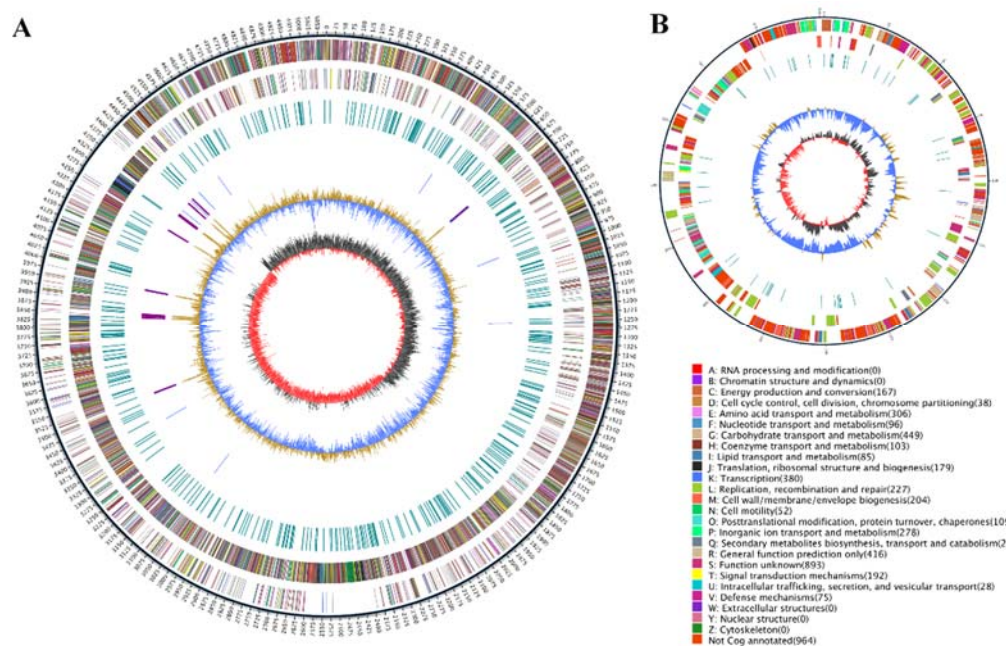


Figure 1. Complete genome circle map of *N. circulans* SWUN-Y10 (A) and Plasmid (B).

Note: the information corresponding to the outer to inner circle is coding genes, gene function annotation results, ncRNA and GC content.

Table 1. Characteristics of *N. circulans* SWUN-Y10 genome

Attributes	Values
Genome Size(bp)	5074781
G+C Content(%)	35.41
Coding Gene Number	5269
Coding Gene Average Length (bp)	857
Total repetitive sequence length (bp)	18765
Plasmid (bp)	401667
rRNA	33
5S rRNA	11
16S rRNA	11
23S rRNA	11
tRNA	83

3.2 Gene annotation of *Niallia circulans* SWUN-Y10

3.2.1 GO database

The GO database, with its broad applicability across diverse organisms, effectively elucidates the functions of genes and proteins through continuous updates and enhancements in its standards (Zhang et al., 2025). The genome of *N. circulans* SWUN-Y10, comprising 3851 genes, was annotated through the Gene Ontology (GO) database. Gene Ontology (GO) terms were assigned to three main domains: cellular component (CC), molecular function (MF), and biological process (BP). (Fig.2A). Annotations derived from the Gene Ontology (GO) database elucidate the functional associations of genes. As shown in Fig.2A, there are 4815 genes related to cells, 4534 genes related to molecular functions, and 6223 genes involved in biological process. In the class of biological process, the number of biological process is the largest. In the class of CCs, the numbers of membranes was the largest. In the class of MFs, the numbers of catalytic activity was the largest. In the class of BPs, the number of metabolic process related genes was the largest.

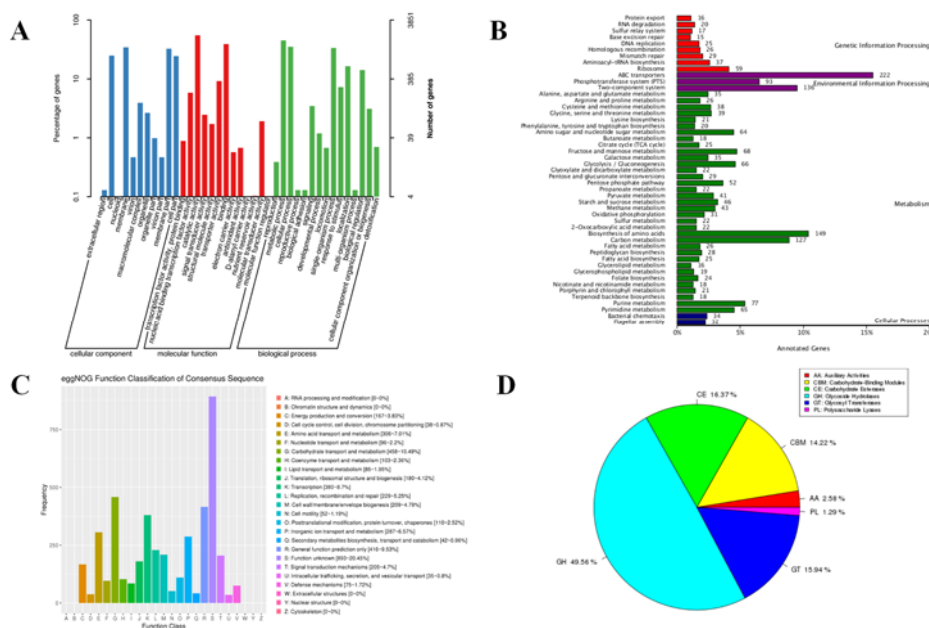


Figure 2. Gene annotation of *N. circularis* SWUN-Y10. (A) GO database annotation. (B) KEGG database annotation. (C) eggNOG database annotation. (D) CAZy database annotation.

3.2.2 KEGG database

The KEGG database encompasses data on biological genomes, metabolic pathways, and associated compounds, with the goal of elucidating the relationships between functional genes and biomolecules at the molecular level (Kanehisa et al., 2023). After annotation with the KEGG database, the metabolic pathways are categorized into four distinct groups. Metabolism is the most prevalent category with 1398 genes, followed by environmental information processing (451 genes), genetic information processing (244 genes) and cellular processes (66 genes). In the Metabolism category, the four pathways with the highest gene counts are: biosynthesis of amino acids (149 genes), carbon metabolism (127 genes), purine metabolism (77 genes) and fructose and mannose metabolism (68 genes). In addition, ABC transporters was particularly abundant in the Environmental Information Processing category, with 222 genes. The above results indicate that *N. circularis* SWUN-Y10 exhibits a robust capacity for biosynthesis of amino acids, material transportation, and environmental adaptability.

To identify genes associated with biogenic amine (BA) synthesis in the genome of SWUN-Y10, a search was conducted using annotations from the KEGG database. The results are presented in Table S1. The results showed SWUN-Y10 lacked genes encoding for critical enzymes like tyrosine decarboxylase (*tyrDC*), histidine decarboxylase (*hdcA*), arginine decarboxylase (*speA*), lysine decarboxylase (*ldcC*), and ornithine decarboxylase (*oaz1*). The absence of a complete enzyme system for synthesis in SWUN-Y10 prevents it from producing the corresponding biogenic amines (Xiong et al., 2024).

3.2.3 EggNOG database

EggNOG, a database constructed through the comparison of protein sequences from complete genomes, is widely utilized for the classification and annotation of genes in newly sequenced genomes (Hernández-Plaza et al., 2023). The annotation of 4366 genes of *N. circularis* SWUN-Y10 into 25 functional

categories was conducted using the eggNOG database. Among them, 458 genes are annotated to Carbohydrate transport and metabolism (G), 306 genes are annotated to in Amino acid transport and metabolism (E), 229 genes are annotated to Replication, recombination and repair, 205 genes are notated to Signal transduction mechanisms (T) and 209 genes are annotated to Cell wall/membrane/envelope biogenesis. The above results exhibited that *N. circulans* SWUN-Y10 has robust capacity of carbohydrate transport and metabolism, proliferation and stress tolerance (Liu et al., 2025).

3.2.4 CAZy database

CAZy, a comprehensive database dedicated to the classification and functional annotation of carbohydrate-active enzymes, is widely used for identifying and characterizing genes involved in carbohydrate metabolism across diverse organisms (Drula et al., 2022). The analysis of carbohydrate-active enzymes (CAZymes) identified 232 genes from six CAZyme gene families within the genome of SWUN-Y10. These CAZymes play a crucial role in sugar metabolism, encompassing processes such as hydrolysis and the formation of glycosidic bonds (Megur et al., 2025). The CAZyme gene families identified in SWUN-Y10 encompass carbohydrate-binding module (CBM) genes, carbohydrate esterase (CE) genes, glycoside hydrolase (GH) genes, auxiliary activity (AA) genes, polysaccharide lyase (PL) genes, and glycosyltransferase (GT) genes. Glycoside hydrolases (GHs) are widespread natural enzymes that possess the capability to break down the most abundant biomasses (Ameri et al., 2022). GH2, GH3 and GH32 which were found in SWUN-Y10 are important members of the glycoside hydrolase family, which play a key role in the degradation of oligosaccharides, breaking down complex carbohydrates into simpler sugars. The activity of these enzymes contributes to the generation of oligosaccharides that can be used as prebiotics (Kim et al., 2024). In addition, SWUN-Y10 contained CBMs. CBMs are frequently linked to GHs and play a crucial role in enhancing the catalytic efficiency of CAZymes by attaching to carbohydrate ligands (Sidar et al., 2020). These findings emphasize that SWUN-Y10 promotes prebiotic formation and its own growth by efficiently metabolizing a range of carbohydrates.

3.3 Antibiotic susceptibility

There are several main mechanisms of bacterial resistance: inherent structural or, and functional properties of bacteria; lack of sensitive target deletion of bacteria to specific antibiotics; mutation or modification of genes of target proteins targeted by antibiotics in bacteria; bacteria degrade or modify antibiotics (Jian et al., 2021). Currently, the increasing prevalence of bacterial resistance to antibiotics is presenting a significant threat to the health of both animals and humans (Saroj & Gupta, 2020). The Comprehensive Antibiotic Resistance Database (CARD) encompasses detailed information on antibiotics and their targets, including antibiotic resistance genes, associated proteins, and mechanisms of resistance (Jia et al., 2016). Based on the CARD database analysis of antibiotic resistance genes in *N. circulans* SWUN-Y10, only one gene of *vmr* was observed (Table S2). *Vmr* is an ABC-F ATPase ribosomal protection protein identified in *Bacillus subtilis*. BLAST alignment showed *vmr* resides on the main chromosome, not the

plasmid. It confers resistance to lincomycin and streptogramin A (virginiamycin) (Crowe-McAuliffe et al., 2018).

As indicated in Table 2, *N. circulans* SWUN-Y10 exhibits drug resistance to lincomycin, with a sensitivity classification of "R". This finding is consistent with the analysis results from the CARD database. The antibiotic susceptibility profiling of *N. circulans* SWUN-Y10 (Table 2) revealed phenotypic evidence demonstrating susceptibility to the majority of clinically relevant antimicrobial agents. *N. circulans* SWUN-Y10 shows high sensitivity to a wide range of antibiotics, thus reducing the risk of spreading antibiotic resistance in the host, making it potentially advantageous in terms of safety as a probiotic (Ye & Chen, 2023). Microbial strains harboring antibiotic resistance plasmids are deemed inappropriate for use as probiotics in humans or animals (Zhou et al., 2024). The presence of the *vmI*R gene on the chromosome of *N. circulans* SWUN-Y10 suggests that this strain is safe and does not carry the risk of plasmid-mediated transmission.

Table 2. Judgement of antibiotic susceptibility level of *N. circulans* SWUN-Y10

Classification	Name	Dosage (ug)	Standard			Bacteriostatic zone diameter/mm	Degree of sensitivity
			S	I	R		
Penicillins	Penicillin	10	≥18	15-17	≤14	49.87 ± 0.91	S
Cephalosporins	Ampicillin	10	≥17	14-16	≤13	47.76 ± 0.40	S
	Gentamicin	10	≥15	13-14	≤12	29.13 ± 0.43	S
Aminoglycosides	Streptomycin	10	≥15	12-14	≤11	18.73 ± 0.41	S
	Kanamycin	30	≥18	14-17	≤13	27.74 ± 0.94	S
Tetracyclines	Tetracycline	30	≥15	12-14	≤11	51.95 ± 0.70	S
	Doxycycline	30	≥14	11-13	≤10	27.06 ± 0.92	S
Phenoxymethylpenicillins	Ciprofloxacin	5	≥26	22-25	≤21	43.17 ± 0.95	S
	Norfloxacin	10	≥17	13-16	≤12	22.49 ± 0.43	S
Macrolides	Erythromycin	15	≥18	15-17	≤14	21.80 ± 0.78	I
	Roxithromycin	15	≥23	14-22	≤13	23.10 ± 0.18	S
Glycopeptides	Vancomycin	30	≥17	15-16	≤14	25.58 ± 0.59	S
Chloramphenicols	Chloramphenicol	30	≥18	13-17	≤12	27.41 ± 2.01	S
Lincosamides	Clindamycin	2	≥21	15-20	≤14	46.48 ± 0.76	S
	Lincomycin	2	≥21	14-21	≤14	0	R
Carbapenems	Imipenem	10	≥16	13-16	≤13	37.25 ± 1.84	S

Note: R indicates drug resistance; I indicates moderately sensitive; S indicates sensitive.

3.4 Hemolytic activity

The utilization of probiotics hinges crucially on their safety in the food industry. A key requirement for incorporating probiotics into food or nutraceutical products is ensuring their secure and harmless nature (Xu et al., 2025). Hemolysis testing plays a critical role in assessing the safety of potential probiotic candidates, ensuring their compatibility with host cells and excluding pathogenic traits associated with erythrocyte lysis (Liang et al., 2024a). The hemolysis of *N. circulans* SWUN-Y10, methicillin-resistant *Staphylococcus aureus* ATCC 43300 (Positive control) and blank TSB broth (Negative control) is depicted in Fig.3A. A distinct β-hemolytic zone was observed surrounding *S. aureus* colonies, whereas *N. circulans* SWUN-Y10 demonstrated no detectable hemolytic activity adjacent to its colonies. Comparative genomic analysis of *N.*

circulans SWUN-Y10 against the Virulence Factor Database (VFDB) is summarized in Table S3 (Supplementary Materials). The genome of *N. circulans* SWUN-Y10 lacked detectable genes for haemolysin BL (Hbl), non-haemolytic enterotoxin (Nhe), enterotoxin (cytotoxin K), and emetic toxin (cereulide - Ces), as determined by VFDB analysis. Analysis of the *N. circulans* SWUN-Y10 genome using the Virulence Factor Database (VFDB) revealed the absence of genes encoding key virulence factors, including haemolysin BL (Hbl), non-haemolytic enterotoxin (Nhe), cytotoxin K, and the emetic toxin cereulide (Ces). In addition, virulence genes identified in *N. circulans* SWUN-Y10 have not been previously reported to be associated with the pathogenicity of *N. circulans*.

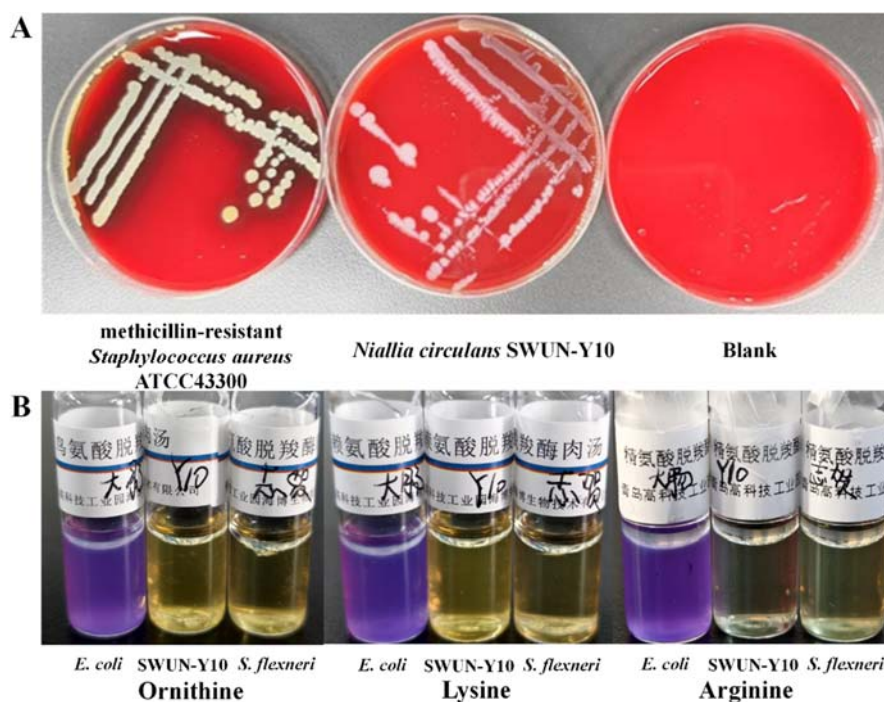


Figure 3. The detection results of harmful metabolites of *N. circulans* SWUN-Y10. (A) Hemolysis activity. (B) Amino decarboxylase test.

3.5 Amino acid decarboxylase test

Bioamines are low-molecular-weight organic compounds containing nitrogen, which are present in plants, animals, and microorganisms. The presence of bioamines in food is associated with food quality and safety. Excessive exposure to bioamines and/or inadequate detoxification capacity can lead to acute toxic effects on the human body (Barbieri et al., 2019; Dabadé et al., 2021). The activity of amino acid decarboxylase in *N. circulans* SWUN-Y10 was assessed using a decarboxylase assay based on the principle that amines alkalize the medium, resulting in a color change from yellow to purple as indicated by bromocresol violet. *Escherichia coli* ATCC25922 (*E. coli*), a biogenic amine producer, served as the positive control, while *Shigella flexneri* CICC 11868 (*S. flexneri*), which does not produce biogenic amines, acted as the negative control. As demonstrated in Fig.3B, the amino acid decarboxylase activity in SWUN-Y10 was negative. The combined analysis of biogenic amine-related genes in *N. circulans* SWUN-Y10 (Section 3.2.2), consistent with the experimental results presented here, demonstrates that this strain lacks the capacity to produce biogenic amines.

3.6 Assessment of probiotic properties

3.6.1 Acid and bile tolerance of *N. circulans* SWUN-Y10

Survival through the low gastric pH and subsequent intestinal bile salts is a fundamental prerequisite for probiotics to colonize and function within the host (Wang et al., 2025). The gastric environment is typically highly acidic (pH 2.0-3.0), while intestinal bile salt concentrations generally range from 0.03% to 0.3% (Wang et al., 2019). Thus, acid resistance in bacillus is critical both for the bacteria's survival and for the successful fermentation and manufacturing of probiotic products. The survivability of *N. circulans* SWUN-Y10 was significantly affected by the medium pH ($P < 0.05$) (Fig.4A). As the medium pH decreased from 6 to 2, there was a significant reduction in bacterial survival rate, dropping from 98.09% to 12.59%. Similarly, *Levilactobacillus brevis* HL6 demonstrated an ability to thrive in highly acidic conditions (pH 2.5~4.5) (Alizadeh Behbahani et al., 2024). This suggests it possesses a natural resistance to the acidic environment of the human gastrointestinal tract.

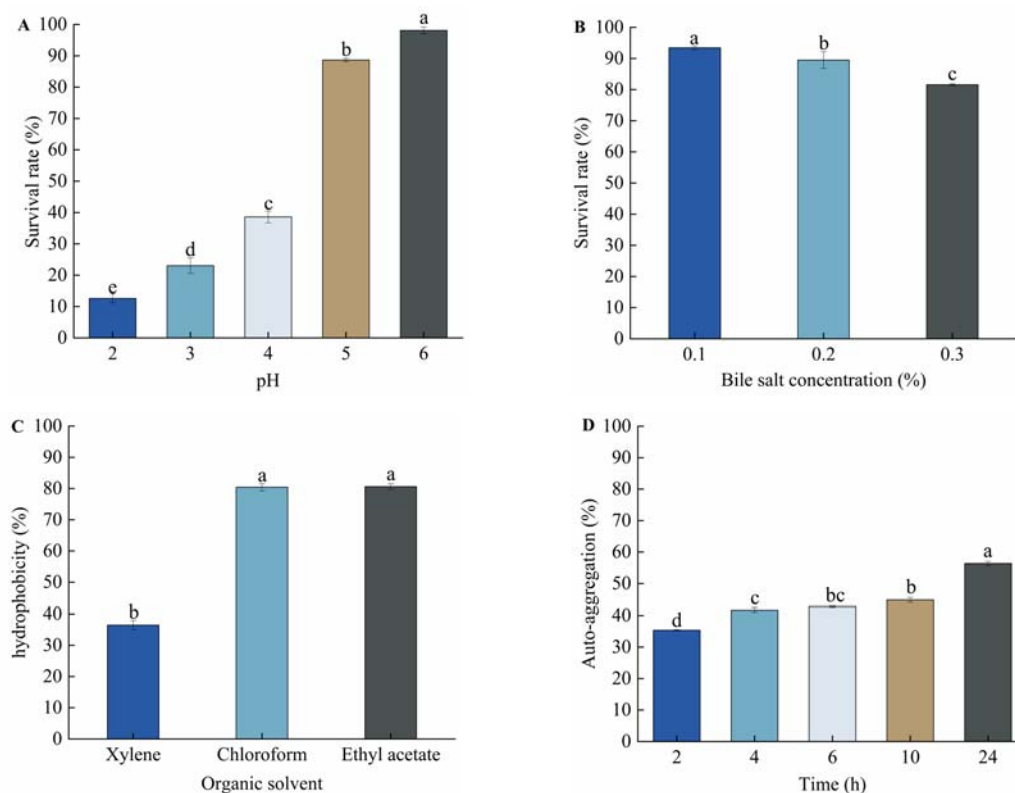


Figure 4. The probiotic properties assessment of *N. circulans* SWUN-Y10 in vitro

(A) SWUN-Y10 survival rate under diverse pH conditions; (B) SWUN-Y10 survival rate under different bile salt concentrations; (C) SWUN-Y10 surface hydrophobicity towards various solvents; (D) SWUN-Y10 auto-aggregation (%) at 2, 4, 6, 10 and 24 h of culture. The bar graph shows the mean $\pm$ SD from three independent trials. Inter-group differences were assessed using one-way ANOVA, and Tukey's multiple range test was carried out for significant analysis. Different letters represent significant differences ($P < 0.05$).

3.7 Acute toxicity

Excessive bile salts compromise the integrity of bacterial cell membranes, ultimately resulting in probiotic cell lysis (Yang et al., 2022). Fig.4B demonstrates a significant decrease in bacterial survival with increasing concentrations of bile salts in the culture medium. However, *N. circulans* SWUN-Y10 maintained a high survival rate of 81.58% even at a bile salt concentration of 0.3%. The notable stability of *N. circulans*

SWUN-Y10 may be attributed to its ability to deconjugate bile salts into cholesterol and amino acids (Zibaei-Rad et al., 2023). *N.circulans* SWUN-Y10 demonstrated notable tolerance to both highly acidic (pH 2.0) and high bile salt (0.3%) conditions, underscoring its potential as a probiotic capable of withstanding the host's digestive tract.

3.6.2 Cell surface hydrophobicity and auto-aggregation of *N. circulans* SWUN-Y10

The efficacy of probiotics depends on their ability to successfully adhere to the host's gastrointestinal tract (Liang et al., 2024b). The adhesion capability can be indirectly assessed by measuring cell surface hydrophobicity and auto-aggregation properties (Zibaei-Rad et al., 2023). Probiotics with high cell surface hydrophobicity (CSH) and strong auto-aggregation ability were correlated with enhanced intestinal adhesion and colonization (Li & So, 2021). Evaluation of bacterial adhesion properties using organic solvents revealed that *N. circulans* SWUN-Y10 exhibited significant hydrophobicity, with rates greater than 80% in chloroform and ethyl acetate (Fig.4C). The adhesion capacity of *N. circulans* SWUN-Y10 increased from an initial 35.36% (at 2 h) to a significantly higher 56.39% after 24 h of incubation (Fig.4D). This result demonstrated superior adhesion compared to the six chicken-derived lactic acid bacteria strains (13.61~30.17%) (Wang et al., 2022). The auto-aggregation capacity of bacteria stems from key surface properties such as charge, proteinaceous structures, and extracellular polysaccharides (Su et al., 2023; Zhang et al., 2022). High auto-aggregation capacity in bacterial strains is considered advantageous because it promotes gut biofilm formation, thereby enhancing their competitive exclusion of pathogens (Gao et al., 2026).

3.7.1 Effect of *N. circulans* SWUN-Y10 on mice body weight, feed intake and organ index

Acute oral toxicity testing constitutes a fundamental component of the safety assessment process for probiotic bacterial strains (Ma et al., 2025). In this study, the safety of the strain SWUN-Y10 was evaluated using acute oral toxicity tests in Balb/c mice. Throughout the entire experimental period, the mice exhibited sleek fur, maintained normal activity levels, and remained free of mortality. As shown in Fig.5A, the body weights of both the control and experimental groups exhibited a consistent upward trend, indicating that the mice were developing normally with no adverse effects observed. In addition, there was no significant difference in food intake and organ indexes between the *N. circulans* SWUN-Y10 group and the control group ($P > 0.05$) (Fig.5B-G). Based on the results, the approximate lethal dose (ALD) of SWUN-Y10 in Balb/c mice following a single oral administration exceeded 1×10^{11} CFU/kg body weight.

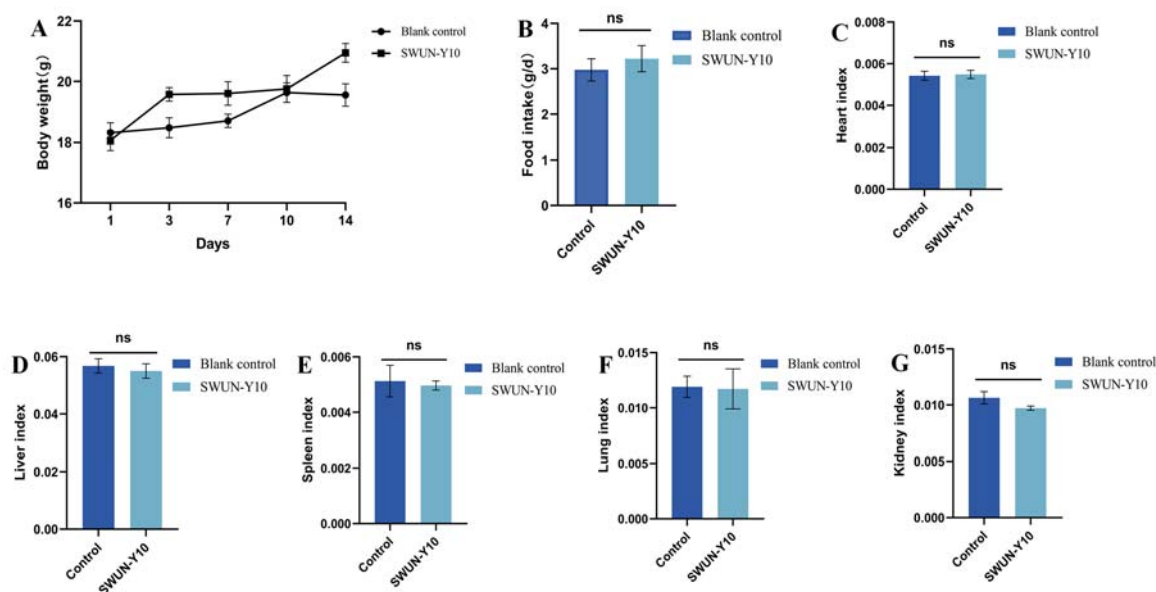


Figure 5. Influence of *N. circulans* SWUN-Y10 on mice body weight, feed intake and organ index.

(A) average body weight; (B) average feed intake; (C) heart index; (D) liver index; (E) spleen index; (F) lung index; (G) kidney index. Results are shown as mean $\pm$ SEM (n=6).

3.7.2 Routine blood indicators

In safety assessments, monitoring routine blood parameters serves to detect potential hematological side effects or metabolic imbalances caused by the administered strain (Yakabe et al., 2009). As shown in Table 3, the hematological parameters of mice administered *N. circulans* SWUN-Y10 showed an increase in Lymph%, RBC, and HGB, and a decrease in Gran and Mon% compared with the control group; however, the differences were not statistically significant ($P > 0.05$). The dataset for RBC, MCV, MCH, and MCHC allows for correlation analysis between erythrocyte size and internal hemoglobin concentration. These parameters are critical for characterizing different degrees of anemia (Silva-Santana et al., 2020). The unchanged RBC indices (MCV, MCH, and MCHC) in the *N. circulans* SWUN-Y10 group compared to the control demonstrate that the strain is harmless to key erythrocyte parameters. The remaining indices showed no significant differences between the *N. circulans* SWUN-Y10-treated group and the control group ($P > 0.05$).

Table 3. Hematological parameters analysis of mice fed of *N. circulans* SWUN-Y10

Indicator parameters	Blank control group	<i>Niallia circulans</i> SWUN-Y10 gavage group
WBC ($10^9/L$)	1.25 ± 0.21^a	1.30 ± 0.14^a
Lymph ($10^9/L$)	1.10 ± 0.07^a	1.20 ± 0.14^a
Mon ($10^9/L$)	0 ± 0^a	0 ± 0^a
Gran ($10^9/L$)	0.18 ± 0.14^a	0.10 ± 0.10^a
Lymph (%)	83.20 ± 15.13^a	90.65 ± 2.76^a
Mon (%)	2.15 ± 1.34^a	1.6 ± 0.28^a
Gran (%)	24.65 ± 13.79^a	18.75 ± 2.47^a
RBC ($10^{12}/L$)	8.155 ± 0.46^a	8.69 ± 0.30^a
HGB (g/L)	137.5 ± 10.71^a	142.5 ± 8.71^a
HCT (%)	32.7 ± 2.28^a	35.85 ± 1.92^a
MCV (fL)	41.15 ± 5.07^a	41.35 ± 3.35^a
MCH (pg)	17.25 ± 0.07^a	17.25 ± 0.49^a
MCHC (g/L)	420 ± 1.41^a	419.5 ± 9.19^a
RDW (%)	11.6 ± 0.1^a	11.6 ± 0.57^a

PLT (10 ⁹ /L)	479 ± 9.8 ^a	485 ± 1.21 ^a
MPV (fL)	4.8 ± 0.28 ^a	4.85 ± 0.07 ^a
PDW	16.1 ± 1.14 ^a	16.5 ± 2.16 ^a
PCT (%)	0.229 ± 0.07 ^a	0.287 ± 0.1 ^a

Note: Data are expressed as mean ± SEM (n = 6). Significant differences were analyzed by One-Way ANOVA, and there were no significant differences between groups ($P > 0.05$). WBC: White blood cells; Lymph: **Lymphocytes**; Mon: Monocytes; Gran: Granulocytes; RBC: Red blood cells; HGB: Hemoglobin concentration; HCT: Hematocrit value; MCV: Mean corpuscular value; MCH: mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red blood cell distribution width; PLT: Platelets; MPV: Mean platelet volume; PDW: platelet distribution width; PCT: Platelet crit.

In addition, serum biochemistry was analyzed to assess toxicity, including key enzymes (ALT, AST), TP, ALB, UREA, GLU, TC, and TG. As shown in Table 4, the experimental groups revealed no significant changes relative to the blank control group ($P > 0.05$). Consistent with the present study, the evaluation by Fan et al (Fan et al., 2023) found no evidence of adverse reactions in the blood routines of mice treated with *N. circulans* SWUN-Y10. The absence of significant adverse effects in the routine blood indices indicates that *N. circulans* SWUN-Y10 is non-toxic to the blood of mice.

Table 4. The serum biochemical indexes analysis of mice

Indicator parameters	Blank control group	<i>Niallia circulans</i> SWUN-Y10 gavage group
ALT (U/L)	25.95 ± 3.04 ^a	24.05 ± 1.34 ^a
AST (U/L)	84.95 ± 8.07 ^a	92.25 ± 9.84 ^a
TP (g/L)	46.45 ± 0.21 ^a	45.35 ± 0.78 ^a
ALB (g/L)	28.45 ± 0.64 ^a	27.7 ± 0.99 ^a
UREA (mmol/L)	7.505 ± 0.49 ^a	7.085 ± 0.09 ^a
GLU (mmol/L)	6.875 ± 1.15 ^a	5.565 ± 0.37 ^a
TC (mmol/L)	2.095 ± 0.16 ^a	2.035 ± 0.18 ^a
TG (mmol/L)	1.11 ± 0.23 ^a	1.185 ± 0.37 ^a

3.7.3 Bacterial translocation detection in vivo

Abnormal bacterial translocation may lead to serious pathological conditions such as sepsis, endocarditis, or bacteremia (Linh et al., 2022). Furthermore, bacterial translocation can displace commensal organisms to new body sites—a phenomenon that can lead to their successful engraftment and subsequent adverse health effects in the new environment (Jin et al., 2022). Therefore, an assessment of the migration potential of *N. circulans* SWUN-Y10 is essential. As shown in Table 5, no growth of *N. circulans* SWUN-Y10 was observed on TSA plates after a 2-day incubation of organ tissue samples from both the experimental and control groups, indicating that the tissues of the experimental animals were not contaminated by *N. circulans* SWUN-Y10. For most opportunistic pathogens, translocation across the epithelial barrier is required to initiate pathogenesis (Jia et al., 2011). In immunocompromised hosts, these translocated commensal bacteria can survive, proliferate, and enter the bloodstream, ultimately causing infection (Donald et al., 2022). In contrast, our findings demonstrate that *N. circulans* SWUN-Y10 did not undergo bacterial translocation, preliminarily indicating its safety as a probiotic strain.

Table 5. Translocation rate of *N. circulans* SWUN-Y10 to liver, spleen, kidney, and heart in mice by oral intragastric administration.

Organization Name	Blank control group	<i>Niallia circulans</i> SWUN-Y10 gavage group
-------------------	---------------------	--

Liver	0/10	0/10
Spleen	0/10	0/10
Kidney	0/10	0/10
Heart	0/10	0/10

3.8 Histopathology

The functional integrity of the liver is a key determinant of a mouse's overall physiological well-being (Wu et al., 2021). Macroscopic examination showed unremarkable liver size and appearance in all mice. Furthermore, histological examination confirmed the structural integrity of the liver lobules in both control and treated groups, and liver cell nuclei were clear and located in the middle of cell (Fig.6). Histological analysis by Wang et al (Wang et al., 2021) revealed no evidence of inflammation, atrophy, or ulceration in the organs of gavaged mice, in agreement with our findings. The results showed that the health of the mice was not adversely affected, even by high-dose administration of *N. circulans* SWUN-Y10.

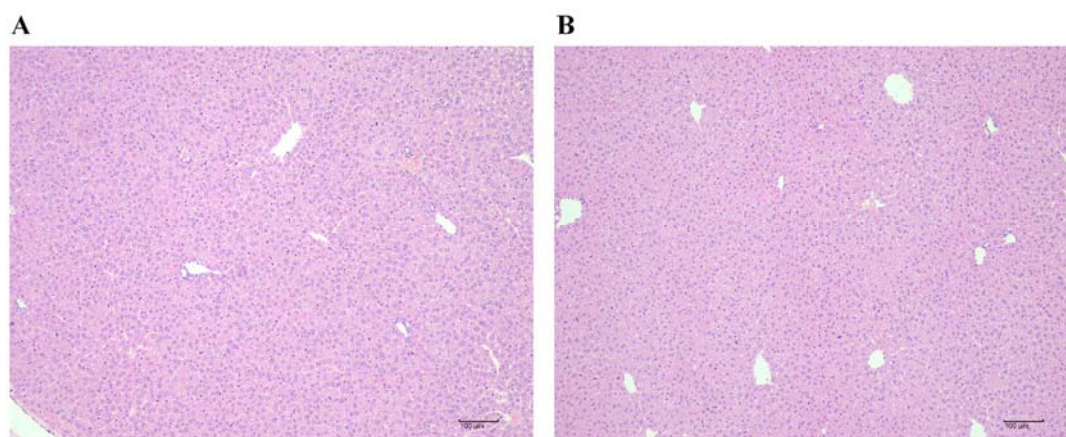


Figure 6. Images of H&E stained liver sections

(A) liver of female mice with control; (B) liver of female mice fed with *N. circulans* SWUN-Y10 (1×10^{11} CFU/kg body weight).

4. Conclusion

This study aimed to excavate the probiotic potential of *N. circulans* SWUN-Y10 by evaluating its key probiotic characteristics and conducting a comprehensive safety assessment using both in vitro and in vivo methods. It exhibited notable functional properties, including robust tolerance to gastric acid and bile salts, high cell surface hydrophobicity, and auto-aggregation capacity, which are crucial for survival and colonization in the gastrointestinal tract. The genomic analysis indicated an absence of genes encoding critical virulence factors. In addition, only a single active antibiotic resistance gene (*vmrR*) was identified. Further safety evaluation through hemolytic activity, antibiotic susceptibility, and biogenic amine production demonstrated a favorable profile. In vivo, no significant effects of *N. circulans* SWUN-Y10 were observed on the normal growth, hemoglobin levels, erythrocyte and leukocyte counts, or other routine blood parameters in mice. Moreover, no evidence of infection was observed, and H&E staining revealed no discernible hepatic injury. The integration of phenotypic and genotypic analyses has elucidated the probiotic potential of *N. circulans* SWUN-Y10, forming a scientific foundation for its development either as a starter culture for food fermentation or as a probiotic for gut health.

Declaration of competing interest

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

This research was supported by the Fundamental Research Funds for the Central Universities, Southwest Minzu University (ZYN2025243) and the WeiZhou Team Funds, Southwest Minzu University (SMUWZ202409).

Data availability

Data will be made available on request.

References

- Ameri R, García JL, Derenfed AB, Pradel N, Neifar S, Mhiri S, Mezghanni M, Jaouadi NZ, Barriuso J, Bejar S. (2022). Genome sequence and Carbohydrate Active Enzymes (CAZymes) repertoire of the thermophilic *Caldicoprobacter algeriensis* TH7C1T. *Microbial Cell Factories*, 21(1), 91. <https://doi.org/10.1186/s12934-022-01818-0>
- Barbieri F, Montanari C, Gardini F, Tabanelli G. (2019). Biogenic amine production by lactic acid bacteria: A review. *Foods*, 8(1), 17. <https://doi.org/10.3390/foods8010017>
- Behbahani, B. A., Jooyandeh, H., Hojjati, M., & Sheikhjan, M. G. (2024). Evaluation of probiotic, safety, and anti-pathogenic properties of *Levilactobacillus brevis* HL6, and its potential application as bio-preservatives in peach juice. *LWT-Food Science and Technology*, 191, 115601. <https://doi.org/https://doi.org/10.1016/j.lwt.2023.115601>
- Bhatia A, Sharma D, Mehta J, Kumarasamy V, Begum MY, Siddiqua A, Sekar M, Subramaniyan V, Wong LS, Mat Rani NNI. (2025). Probiotics and synbiotics: applications, benefits, and mechanisms for the improvement of human and ecological health. *Journal of Multidisciplinary Healthcare*, 1493-1510. <https://doi.org/10.2147/jmdh.S501056>
- Cheng X, Tan Y, Li H, Huang J, Zhao D, Zhang Z, Yi M, Zhu L, Hui S, Yang J, Peng W. (2022). Fecal 16S rRNA sequencing and multi-compartment metabolomics revealed gut microbiota and metabolites interactions in APP/PS1 mice. *Computers in Biology and Medicine*, 151(Pt A):106312. <https://doi.org/10.1016/j.compbimed.2022.106312>
- Crowe-McAuliffe C, Graf M, Huter P, Takada H, Abdelshahid M, Nováček J, Murina V, Atkinson GC, Hauryliuk V, Wilson DN. (2018). Structural basis for antibiotic resistance mediated by the *Bacillus subtilis* ABCF ATPase VmlR. *Proceedings of the National Academy of Sciences*, 115(36), 8978-8983. <https://doi.org/10.1073/pnas.1808535115>
- Cui Y, Zhu J, Li P, Guo F, Yang B, Su X, Zhou H, Zhu K, Xu F. (2024). Assessment of probiotic *Bacillus velezensis* supplementation to reduce *Campylobacter jejuni* colonization in chickens. *Poultry Science*, 103(8), 103897. <https://doi.org/https://doi.org/10.1016/j.psj.2024.103897>
- Dabadé, D. S., Jacxsens, L., Miclotte, L., Abatih, E., Devlieghere, F., & De Meulenaer, B. (2021). Survey of multiple biogenic amines and correlation to microbiological quality and free amino acids in foods. *Food Control*, 120, 107497. <https://doi.org/https://doi.org/10.1016/j.foodcont.2020.107497>
- Donald K, Petersen C, Turvey SE, Finlay BB, Azad MB. (2022). Secretory IgA: Linking microbes, maternal health, and infant health through human milk. *Cell Host & Microbe*, 30(5), 650-659. <https://doi.org/https://doi.org/10.1016/j.chom.2022.02.005>
- Drula E, Garron ML, Dogan S, Lombard V, Henrissat B, Terrapon N. (2022). The carbohydrate-active enzyme database: functions and literature. *Nucleic Acids Research*, 50 (D1), D571-D577. <https://doi.org/10.1093/nar/gkab1045>
- Duche RT, Singh A, Wandhare AG, Sangwan V, Sihag MK, Nwagu TNT, Panwar H, Ezeogu LI. (2023). Antibiotic resistance in potential probiotic lactic acid bacteria of fermented foods and human origin from Nigeria. *BMC Microbiology*, 23(1), 142. <https://doi.org/10.1186/s12866-023-02883-0>

- Fan, X., Jiang, X., Guo, Y., Zhang, T., Zeng, X., Wu, Z., & Pan, D. (2023). In vitro and in vivo evaluation of the safety of *Levilactobacillus brevis* CGMCC1.5954 with probiotic potential based on tri-generation whole genome sequencing and animal studies. *Food Bioscience*, 53, 102654. <https://doi.org/https://doi.org/10.1016/j.fbio.2023.102654>
- Feng, J., Du, X., Zhang, Y., Xie, Y., Cong, Y., He, J., & Zhang, W. (2025). Isolation and characterization of *Bacillus velezensis* YM1 and its mechanism for zearalenone degradation. *LWT-Food Science and Technology*, 224, 117810. <https://doi.org/https://doi.org/10.1016/j.lwt.2025.117810>
- Freire, V., Condón, S., & Gayán, E. (2024). Impact of sporulation temperature on germination of *Bacillus subtilis* spores under optimal and adverse environmental conditions. *Food Research International*, 182, 114064. <https://doi.org/https://doi.org/10.1016/j.foodres.2024.114064>
- Gao, W., Hernandez-Garcia, L., Castagnini, J. M., Pallares, N., Barba, F. J., & Culebras, P. V. M. (2026). Identification, safety evaluation and probiotic potential of lactic acid bacteria isolated from wheat. *International Journal of Food Microbiology*, 444, 111436. <https://doi.org/https://doi.org/10.1016/j.ijfoodmicro.2025.111436>
- Gu, J., Song, K., Fan, Y., Dong, Y., Qian, L., & Gai, Z. (2024). Safety evaluation of *Bifidobacterium animalis* subsp. *lactis* BLa80 under in vitro, and in vivo conditions. *Microbial Pathogenesis*, 194, 106809. <https://doi.org/https://doi.org/10.1016/j.micpath.2024.106809>
- Hernández-Plaza, A., Szklarczyk, D., Botas, J., Cantalapiedra, Carlos P., Giner-Lamia, J., Mende, D. R., Kirsch, R., Rattei, T., Letunic, I., Jensen, L. J., Bork, P., Mering, C., & Huerta-Cepas, J. (2023). eggNOG 6.0: enabling comparative genomics across 12 535 organisms. *Nucleic Acids Research*, 51(D1), D389-D394. <https://doi.org/10.1093/nar/gkac1022>
- Hossain MI, Saleh NUA, Numan A, Hossain MM, Uddin MA, Hossain MS. (2023). *Bombyx mori* as a model for *Niallia circulans* pathogenicity. *Drug Discov Ther.* 17(1):18-25. <https://doi.org/10.5582/ddt.2022.01112>.
- Jia, B., Raphenya, A. R., Alcock, B., Wagglechner, N., Guo, P., Tsang, K. K., Lago, B. A., Dave, B. M., Pereira, S., Sharma, A. N., Doshi, S., Courtot, M., Lo, R., Williams, L. E., Frye, J. G., Elsayegh, T., Sardar, D., Westman, E. L., Pawlowski, A. C., Johnson, T. A., Brinkman, F. S. L., Wright, G. D., & McArthur, A. G. (2016). CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Research*, 45(D1), D566-D573. <https://doi.org/10.1093/nar/gkw1004> %J Nucleic Acids Research
- Jia, X., Wang, W., Song, Y., & Li, N. (2011). A 90-day oral toxicity study on a new strain of *Lactobacillus paracasei* in rats. *Food and Chemical Toxicology*, 49(5), 1148-1151. <https://doi.org/https://doi.org/10.1016/j.fct.2011.02.006>
- Jian, Z., Zeng, L., Xu, T., Sun, S., Yan, S., Yang, L., Huang, Y., Jia, J., & Dou, T. (2021). Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *Journal of basic microbiology*, 61(12), 1049–1070. <https://doi.org/10.1002/jobm.202100201>
- Jin, S., Wetzel, D., & Schirmer, M. (2022). Deciphering mechanisms and implications of bacterial translocation in human health and disease. *Current Opinion in Microbiology*, 67, 102147. <https://doi.org/https://doi.org/10.1016/j.mib.2022.102147>
- Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., & Ishiguro-Watanabe, M. (2023). KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Research*, 51(D1), D587-D592. <https://doi.org/10.1093/nar/gkac963>
- Kim, S., Kang, J. Y., Nguyen, Q. A., & Lee, J. S. (2024). Effects of prebiotic dietary Fibers on the stimulation of the mucin secretion in host cells by in vitro gut microbiome consortia. *Foods (Basel, Switzerland)*, 13(19), 3194. <https://doi.org/10.3390/foods13193194>
- Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Fernández Escámez, P. S., Maradona, M. P., Querol, A., Suarez, J. E., Sundh, I., Vlak, J., Barizzzone, F., Herman, S. C., & Herman, L. (2020). Scientific opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017-2019). EFSA journal. *European Food Safety Authority*, 18(2), e05966. <https://doi.org/10.2903/j.efsa.2020.5966>
- Li B, Cai L, Liao B, Fu X, Bing P, Yang J. (2019). Prediction of Protein Subcellular Localization Based on Fusion of Multi-view Features. *Molecules*, 24(5), 919. <https://doi.org/10.3390/molecules24050919>
- Li M, Wang Y, Cui H, Li Y, Sun Y, Qiu HJ. (2020). Characterization of lactic acid bacteria isolated from the gastrointestinal tract of a wild boar as potential probiotics. *Frontiers in Veterinary Science*, 7:49. <https://doi.org/10.3389/fvets.2020.00049>

- Li, S. & So, J. (2021). In vitro characterization of cell surface properties of 14 vaginal *Lactobacillus* strains as potential probiotics. *Advances in Microbiology*, 11, 144-155. <https://doi.org/10.4236/aim.2021.112010>
- Liaqat I, Qaiser I, Aftab MN, Ali S, Latif AA, Naseem S, Afzaal M, Khalid A. Anti-biofilm potential of some fish probiotics, alone and in combination with antibiotics against isolated aquaculture pathogens; A preliminary data. *Microb Pathog*. 2025; 203, 107437. <https://doi.org/10.1016/j.micpath.2025.107437>.
- Liang, A., Wang, J., Ding, L., Zou, L., Wang, D., Zhu, C., & Tang, J. (2024a). Probiotic properties, whole-genome sequence analysis, and safety assessment of *BreviBacillus borstelensis* S8. *LWT-Food Science and Technology*, 210, 116800. <https://doi.org/10.1016/j.lwt.2024.116800>
- Liang, X., Dai, N., Yang, F., Zhu, H., Zhang, G., & Wang, Y. (2024b). Molecular identification and safety assessment of the potential probiotic strain *Bacillus paralicheniformis* HMPM220325 isolated from artisanal fruit dairy products. *Food Funct*, 15(2), 747-765. <https://doi.org/10.1039/d3fo04625g>
- Linh, H. T., Iwata, Y., Senda, Y., Sakai-Takemori, Y., Nakade, Y., Oshima, M., Nakagawa-Yoneda, S., Ogura, H., Sato, K., Minami, T., Kitajima, S., Toyama, T., Yamamura, Y., Miyagawa, T., Hara, A., Shimizu, M., Furuichi, K., Sakai, N., Yamada, H., Asanuma, K., Matsushima, K., & Wada, T. (2022). Intestinal bacterial translocation contributes to diabetic kidney disease. *Journal of the American Society of Nephrology: JASN*, 33(6), 1105–1119. <https://doi.org/10.1681/asn.2021060843>
- Liu, X., Shi, Y., Liu, D., & Chen, Q. (2025). A novel exopolysaccharide-producing bacterium from traditional rice wine koji in China: Whole genome sequencing, exopolysaccharide isolation and bio-functional properties assessment. *LWT-Food Science and Technology*, 217, 117375. <https://doi.org/https://doi.org/10.1016/j.lwt.2025.117375>
- Lodi, R. S., Jia, X., Yang, P., Peng, C., Dong, X., Han, J., Liu, X., Wan, L., & Peng, L. (2025). Whole genome sequencing and annotations of *Trametes sanguinea* ZHSJ. *Scientific data*, 12 (1), 1460. <https://doi.org/10.1038/s41597-025-05798-9>
- Lu, J., Mao, Y., Ma, T., Liu, X., Cheng, X., Bai, Y., & Li, S. (2023). Screening and genome analysis of lactic acid bacteria with high exopolysaccharide production and good probiotic properties. *Food Bioscience*, 56, 103211. <https://doi.org/10.1016/j.fbio.2023.103211>
- Ma, D., Hou, S., Gong, Y., Wu, S., Hui, Q., & Hao, Z. (2025). Safety evaluation of Areca catechu L. extract powder in rats: acute and 180-day chronic toxicity test. *Journal of Ethnopharmacology*, 350, 119946. <https://doi.org/10.1016/j.jep.2025.119946>
- Mačianskienė, R., Zigmantaitė, V., Andriulė, I., Pangonytė, D., Sadauskienė, I., Arandarčikaitė, O., Stankevičius, A., Grigas, J., Pautienius, A., Treinys, R., Navalinskas, A., Grigalevičiūtė, R., Kučinskas, A., Pudžiuvėlytė, L., Bernatoniene, J., & Jurevičius, J. (2023). Acute and sub-chronic intraperitoneal toxicity studies of the *Elsholtzia ciliata* herbal extract in Balb/c mice. *Pharmaceutics*, 15(10), 2417. <https://doi.org/10.3390/pharmaceutics15102417>
- Megur, A., Ambrutaitytė, K., Šimoliūnas, E., Lastauskienė, E., & Burokas, A. (2025). Whole-genome sequencing and in vitro probiotic characterization of *Pediococcus pentosaceus* ELAB 60WB isolated from fermented cherry tomatoes. *LWT-Food Science and Technology*, 220, 117547. <https://doi.org/https://doi.org/10.1016/j.lwt.2025.117547>
- Mendonça, A. A., Pinto-Neto, W. D. P., da Paixão, G. A., Santos, D. D. S., De Moraes Jr, M. A., & De Souza, R. B. (2022). Journey of the probiotic bacteria: Survival of the fittest. *Microorganisms*, 11(1), 95. <https://doi.org/10.3390/microorganisms11010095>
- Patel, S., & Gupta, R. S. (2020). A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus *Bacillus*: Proposal for six new genera of *Bacillus* species, *Peribacillus* gen. nov., *Cytobacillus* gen. nov., *Mesobacillus* gen. nov., *Neobacillus* gen. nov., *Metabacillus* gen. nov. and *Alkalihalobacillus* gen. nov. *International Journal of Systematic and Evolutionary Microbiology*, 70(1), 406-438. <https://doi.org/10.1099/ijsem.0.003775>
- OECD (2022), Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264071049-en>.
- Sabino, Y. N. V., de Araújo Domingues, K. C., O'Connor, P. M., Marques, P. H., Santos, E. H., Tótola, M. R., Abreu, L. M., de Queiroz, M. V., Cotter, P. D., & Mantovani, H. C. (2024). *Bacillus velezensis* iturins inhibit the hemolytic activity of *Staphylococcus aureus*. *Scientific Reports*, 14(1), 9469. <https://doi.org/10.1038/s41598-024-58973-0>
- Santiya, M., Bhat, M. I., Gupta, T., Kapila, S., & Kapila, R. (2020). Safety assessment of potential probiotic *Lactobacillus fermentum* MTCC-5898 in murine model after repetitive dose for 28 Days (sub-acute exposure). *Probiotics and Antimicrobial Proteins*, 12(1), 259-270. <https://doi.org/10.1007/s12602-019-09529-6>

- Saroj, D. B., & Gupta, A. K. (2020). Genome based safety assessment for *Bacillus coagulans* strain LBSC (DSM 17654) for probiotic application. *International Journal of Food Microbiology*, 318, 108523. <https://doi.org/https://doi.org/10.1016/j.ijfoodmicro.2020.108523>
- Sibanda, T., Marole, T. A., Thomashoff, U. L., Thantsha, M. S., & Buys, E. M. (2024). Bifidobacterium species viability in dairy-based probiotic foods: challenges and innovative approaches for accurate viability determination and monitoring of probiotic functionality. *Frontiers in Microbiology*, 15, 1327010. <https://doi.org/10.3389/fmicb.2024.1327010>
- Sidar, A., Albuquerque, E. D., Voshol, G. P., Ram, A. F. J., Vijgenboom, E., & Punt, P. J. (2020). Carbohydrate binding modules: Diversity of domain architecture in amylases and cellulases from filamentous microorganisms. *Frontiers in bioengineering and biotechnology*, 8, 871. <https://doi.org/10.3389/fbioe.2020.00871>
- Silva-Santana, G., Bax, J. C., Fernandes, D. C. S., Bacellar, D. T. L., Hooper, C., Dias, A. A. S. O., Silva, C. B., de Souza, A. M., Ramos, S., Santos, R. A., Pinto, T. R., Ramão, M. A., & Mattos-Guaraldi, A. L. (2020). Clinical hematological and biochemical parameters in Swiss, BALB/c, C57BL/6 and B6D2F1 Mus musculus. *Animal models and experimental medicine*, 3(4), 304–315. <https://doi.org/10.1002/ame2.12139>
- Soni, R., Keharia, H., Dunlap, C., Pandit, N., & Doshi, J. (2022). Functional annotation unravels probiotic properties of a poultry isolate, *Bacillus velezensis* CGS1. 1. *LWT-Food Science and Technology*, 153, 112471. <https://doi.org/10.1016/j.lwt.2021.112471>
- Su, M., Tang, T., Tang, W., Long, Y., Wang, L., & Liu, M. (2023). Astragalus improves intestinal barrier function and immunity by acting on intestinal microbiota to treat T2DM: a research review. *Frontiers in Immunology*, 14, 1243834. <https://doi.org/10.3389/fimmu.2023.1243834>
- Vijayaganapathi, A., & Mohanasrinivasan, V. (2025a). A review of next-generation probiotics—as a gateway to biotherapeutics. *Probiotics and Antimicrobial Proteins*, 17(4), 1985–1997. <https://doi.org/10.1007/s12602-025-10606-2>
- Vijayaganapathi, A., & Mohanasrinivasan, V. (2025b). Evaluation of probiotic potential, safety assessment and whole genome sequencing of *Lactiplantibacillus plantarum* strain MOVIN isolated from toddy sample. *Frontiers in Microbiology*, 16, 1625659. <https://doi.org/10.3389/fmicb.2025.1625659>
- Wang, D., Wang, Y., Lan, H., Wang, K., Zhao, L., & Hu, Z. (2021). Enhanced production of γ -aminobutyric acid in litchi juice fermented by *Lactobacillus plantarum* HU-C2W. *Food Bioscience*, 42, 101155. <https://doi.org/10.1016/j.fbio.2021.101155>
- Wang, F., Li, X., Wang, Q., Jin, Q., Fu, A., Zhang, Q., Yang, R., Deng, B., & Li, W. (2024). Based on whole genome sequencing and metabolomics analysis the safety and probiotic characteristics of *Lactocaseibacillus paracasei* 36 isolated from Chinese fermented vegetables. *Food Bioscience*, 62, 105405. <https://doi.org/10.1016/j.fbio.2024.105405>
- Wang, H., Mei, L., Deng, Y., Liu, Y., Wei, X., Liu, M., Zhou, J., Ma, H., Zheng, P., Yuan, J., & Li, M. (2019). *Lactobacillus brevis* DM9218 ameliorates fructose-induced hyperuricemia through inosine degradation and manipulation of intestinal dysbiosis. *Nutrition (Burbank, Los Angeles County, Calif.)*, 62, 63–73. <https://doi.org/10.1016/j.nut.2018.11.018>
- Wang, H., Zhang, X., Yu, J., Huang, Y., & Wang, R. (2025). In vitro screening and probiotic properties of lactic acid bacteria with anti-hyperuricemia capacity. *Food Bioscience*, 72, 107511. <https://doi.org/https://doi.org/10.1016/j.fbio.2025.107511>
- Wang, P., Chen, S., Liao, C., Jia, Y., Li, J., Shang, K., Chen, J., Cao, P., Li, W., Li, Y., Yu, Z., & Ding, K. (2022). Probiotic properties of chicken-derived highly adherent lactic acid bacteria and inhibition of enteropathogenic bacteria in Caco-2 Cells. *Microorganisms*, 10(12), 2515. <https://doi.org/10.3390/microorganisms10122515>
- Xiong, J., Feng, L., Yousaf, M., Zeng, S., Tang, J., Wu, Y., Li, Q., & Liu, D.-m. (2024). Assessment of uric acid-lowering activity, safety and stress tolerance of *Lactiplantibacillus plantarum* MC14 based on whole gene sequencing and phenotyping experiments. *Food Bioscience*, 61, 104536. <https://doi.org/10.1016/j.fbio.2024.104536>
- Wu, Z., Wang, P., Pan, D., Zeng, X., Guo, Y., & Zhao, G. (2021). Effect of adzuki bean sprout fermented milk enriched in γ -aminobutyric acid on mild depression in a mouse model. *Journal of Dairy Science*, 104(1), 78–91. <https://doi.org/10.3168/jds.2020-19154>
- Xu, Y., Xiong, T., zhang, L., Du, T., Madjirebaye, P., Zhao, M., & Kang, X. (2025). Novel lactic acid bacteria with anti-hyperglycaemic properties: In vitro screening and probiotic assessment. *Food Bioscience*, 63, 105696. <https://doi.org/10.1016/j.fbio.2024.105696>

- Yakabe, T., Moore, E. L., Yokota, S., Sui, H., Nobuta, Y., Fukao, M., Palmer, H., & Yajima, N. (2009). Safety assessment of *Lactobacillus brevis* KB290 as a probiotic strain. *Food and chemical toxicology*, 47(10), 2450–2453. <https://doi.org/10.1016/j.fct.2009.07.001>
- Yang, X., Yang, J., Ye, Z., Zhang, G., Nie, W., Cheng, H., Peng, M., Zhang, K., Liu, J., Zhang, Z., & Shi, J. (2022). Physiologically inspired mucin coated *Escherichia coli* nissle 1917 enhances biotherapy by regulating the pathological microenvironment to improve intestinal colonization. *ACS nano*, 16(3), 4041–4058. <https://doi.org/10.1021/acsnano.1c09681>
- Ye, J., & Chen, X. (2022). Current promising strategies against antibiotic-resistant bacterial infections. *Antibiotics*, 12(1), 67. <https://doi.org/10.3390/antibiotics12010067>
- Zhang, Q., Xu, B., Min, Y., Liu, J., Shang, Y., Lan, X., Xiang, W., Tang, J. (2025). Evaluation of the safety and probiotic properties of GABA-producing *Enterococcus faecium* AB157 based on whole genome and phenotype analysis. *LWT-Food Science and Technology*, 215, 117242. <https://doi.org/10.1016/j.lwt.2024.117242>
- Zhang, W., Lai, S., Zhou, Z., Yang, J., Liu, H., Zhong, Z., Fu, H., Ren, Z., Shen, L., Cao, S., Deng, L., & Peng, G. (2022). Screening and evaluation of lactic acid bacteria with probiotic potential from local Holstein raw milk. *Frontiers in microbiology*, 13, 918774. <https://doi.org/10.3389/fmicb.2022.918774>
- Zheng, X., Liang, Q., Zhao, B., Song, X., & Zhang, Y. (2024). Whole genome sequencing and analysis of probiotic characteristics for *Lactiplantibacillus plantarum* EL2 isolated from yak yogurt. *LWT-Food Science and Technology*, 198, 116039. <https://doi.org/10.1016/j.lwt.2024.116039>
- Zhou, H., Lu, Z., Liu, X., Bie, X., Xue, F., Tang, S., Feng, Q., Cheng, Y., & Yang, J. (2024). Environmentally relevant concentrations of tetracycline promote horizontal transfer of antimicrobial resistance genes via plasmid-mediated conjugation. *Foods*, 13(11), 1787. <https://doi.org/10.3390/foods13111787>
- Zibaei-Rad, A., Rahmati-Joneidabad, M., Alizadeh Behbahani, B., & Taki, M. (2023). Assessing the protection mechanisms on *Enterobacter aerogenes* ATCC 13048 by potentially probiotic strain *Lacticaseibacillus casei* XN18: An experimental and modeling study. *Microbial Pathogenesis*, 181, 106177. <https://doi.org/10.1016/j.micpath.2023.106177>