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# Genome-based evaluation of safety and probiotic properties of *Lactobacillus plantarum* Lrld-22

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**Abstract**

*Lactobacillus plantarum* is a widely distributed and significant probiotic species. A strain, *L. plantarum* Lrld-22, was isolated from traditional fermented yak dairy products in Qinghai province. The whole genome of Lrld-22 was sequenced, and the sequence result was assembled into a 3,246 Mbp sketch genome. Two plasma sequences of 81,471 and 41,833 bp were detected. The Lrld-22 genome was made up of a single circular chromosome, measuring 3,246,150 bp and containing 3,284 genes, 99 uncoded RNAs, and a number of repetitive sequences. The genome had a GC content of 44.58%, and no prophage region was detected. Functional annotation revealed a large number of the genes involved in carbohydrate and fatty acid metabolisms, suggesting that *L. plantarum* Lrld-22 could have enhanced capabilities for carbohydrate and fat utilisation.

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## Introduction

*Lactobacillus plantarum* is a Gram-positive, non-spore-forming, and facultative anaerobic bacterium (Todorov and Franco, 2010). It exists widely in nature, especially in various fermented foods (Fiocco *et al*., 2010). It enhances the nutritional value, flavour, and food preservation of fermented foods, playing a critical role in the fermented vegetables, beverages, caffeine, cheeses, and meat products (Behera *et al*., 2020; El Sheikha and Hu, 2020). It exhibits significant biological activity, aiding in the regulation of immune function, management of chronic metabolic disorders, defence against pathogenic bacterial infections, and maintenance of intestinal health, among other roles (Cebeci and Gürakan, 2003; Huang *et al*., 2024). It is widely used in foods, feeds, and medications due to its beneficial physiological qualities and probiotic activities (El Sheikha and Hu, 2020).

As the scope of application of *L. plantarum* expands, concerns about its safety have increased, and research on its safety evaluation is also essential. Despite *L. plantarum* having the "generally recognised as safe, GRAS" status, awarded by the United States Food and Drug Administration, USFDA (Seddik *et al*., 2017), it may still develop adverse reactions, such as severe infections and resistant gene transfer (Pradhan *et al*., 2019). While *in vitro* testing remains the primary method for assessing the nature of lactic acid bacteria (LAB), it fails short of addressing the growing need for risk assessment across numerous strains (Bernardeau *et al*., 2008). All-genome sequencing is a high-permeability and effective technical tool for evaluating the safety of LAB, particularly through the analysis of complete genetic information, such as toxicity genes. This approach enables the identification of differences in strain levels, which are important for beneficial effects, and provide guidance and help for effective development of probiotics (Evanovich *et al*., 2019).

The traditional fermented yak dairy products in Qinghai province are rich in a variety of beneficial LABs, including *Streptococcus thermophilus*, *L. fermentum*, and *L. plantarum*. *L. plantarum*, the

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predominant LAB, is naturally present in a variety of environmental ecosystems, some of which are widely used in commercial probiotic cultivation (Mo *et al*., 2019). Although *L. plantarum* has been isolated from fermented plant samples, it is rarely separated from fermented yak dairy and nut yogurt (Pei *et al*., 2018; Shori *et al*., 2022). In the present work, we isolated *L. plantarum* Lrld-22 from traditional fermented yak dairy products, and it demonstrated good fermentation properties in nut-based yogurt made from *Carya cathayensis* Sarg. and *Torreya grandis*. The present work thus aimed to evaluate the genome and probiotic properties of the strain *L. plantarum* Lrld-22.

## Materials and methods

### *Materials*

The traditional fermented yak dairy products were purchased from the supermarket in Qinghai, Xining (Qinghai Little Yak Dairy Co., Ltd., Xining, China). MRS Medium (Hangzhou Microbial Reagent Co., Ltd., Hangzhou, China), Gram-staining Kit (BKMAM Biology Ltd., Changde, China), DNA Extraction Kit (Takara Bio Inc., Shanghai, China), and 2× Taq Plus PCR MasterMix (Takara Bio, Otsu, Japan) were purchased from Hefeimuchen Bioscience Co., Ltd.

### *Methods*

#### *Isolation and cultivation of L. plantarum Lrld-22*

First, 10 mL of sample was diluted with 90 mL of sterile distilled water, and homogenised with high-speed (Ohaus International Trading Co., Ltd., shanghai, China) currents for 30 sec. Next, 10-fold dilution was made with sterile distilled water. Then, 100 μL of each dilution factor was aliquoted onto a Petri dish containing MRS medium, and incubated at 37°C for 24 to 48 h (Liu, 2018). Pure colony was selected for identification and whole genome sequencing.

#### *Identification of L. plantarum Lrld-22*

Identification of *L. plantarum* Lrld-22 was carried out using a combination of morphological and molecular techniques. The colony morphology on MRS medium was observed under a microscope after Gram-staining (El Oirdi *et al*., 2021). The extraction of DNA was conducted following the manufacturer's instructions provided with the Bacteria DNA Kit (Takara Bio Inc., Shanghai, China). Primers 27f and 1492r were used for amplifying the 16S rDNA. The PCR amplification reaction mixture consisted of 2×PCR Master Mix (12.5 μL), upstream and downstream primers, genomic DNA (1 μL each), and DNAse-free water added to achieve a final volume of 25 μL (Wen *et al*., 2016). The reaction conditions were referred to the kit's manufacturer's instruction (Takara Bio, Otsu, Japan). The target bands were detected by electrophoresis of 5 μL PCR products on 1% agarose gel for 10 min, and the amplified products were sent to Shanghai Shenggong Biotechnology Co., Ltd. for sequencing. The sequencing results were compared at the U.S. National Biotechnology Information Centre, and a system development tree was constructed using the tools provided by the platform.

#### *Whole-genome sequencing*

A nanodrop micro-ultraviolet spectrophotometer (Nano-100, Allsheng Instruments, China) was used to measure the amount of DNA present, and the ratios of A260/A280 and A260/A230. 1.5% agar-gel electrophoresis (Beckman Coulter Genomics, Danvers, MA, USA) was used to check the integrity and purity of genomic DNA. After the quality met the requirements for database construction and computer installation, whole-genome sequencing was performed using BGI's BGISEQ platform (BGI Genomics Co., Ltd.) and Nanopore platform (Oxford Nanopore Technologies, Oxford, UK). The BGISEQ procedure involved using a Covaris machine to ultrasonically break the DNA sample to obtain a short DNA fragment of the required length. The Qubit dsDNA HS Assay Kit (Cat No. Q32851; Thermofisher Scientific) tests were run on the cleaned DNA sample, and then the final library was made by PCR, and sequenced. Original reads were obtained through third-generation sequencing of samples using the Nanopore platform. High-quality sequences were obtained after quality control, and assembled through Canu software (v1.5). After assembly, GATK software (3.4-0-g7e26428) was used to correct the third-generation data from the second-generation data. And the Circlator (V1.5.5) software was used to cycle through the fixed three-generation data, and get the full genome sequence of strain Lrld-22 (Accession: PRJNA1081813 ID: 1081813; https://www.ncbi.nlm.nih.gov/bioproject/?term=Lrld-22).

*Comparison of genomic analyses*

*ANI values for average nucleotide identity (ANI)*

The ANI value of strain Lrld-22 was calculated, and the ANI clustering heat map was constructed by FastANI (1.32).

*Construction of phylogenetic tree*

A phylogenetic tree was constructed based on single-copy genes from samples and reference strains. The NJ algorithm TreeBeST (treebest-1.9.2) was used to construct the phylogenetic tree, and the parameter was set to treebest phyml -b 1000 (Nandi *et al*., 2010).

*Functional genome prediction and annotation*

First, the function of the assembled Lrld-22 genome was annotated. Seven databases (KEGG, COG, NR, Swiss-Prot, GO, TrEMBL, and EggNOG) were utilised for generic function annotation. Pathogenicity and medication resistance analyses were available in four databases. A database called ARDB (Antibiotic Resistance Genes Database) and a database called VFDB (Virulence Factors of Pathogenic Bacteria) were used to find virulence factors and resistance genes (Huang *et al*., 2021). EffectiveT3 identified effector proteins of the Type III secretion system.

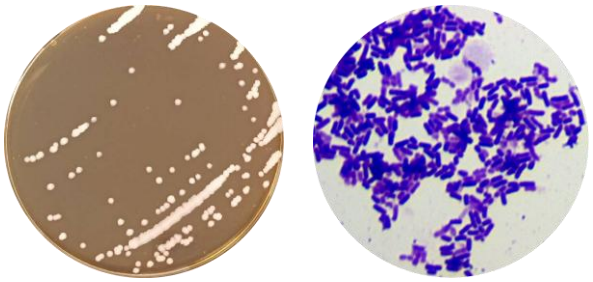
*Safety evaluation of L. plantarum Lrld-22*

The resistance of strain *L. plantarum* Lrld-22 to antibiotics was evaluated by referring to the method reported by Cebeci and Gürakan (2003) and Todorov *et al*. (2017). The growth of strain Lrld-22 was detected at 600 nm using a spectrophotometer 725 (Shanghai Spectrum Instrument Co., Ltd., Shanghai, China), and they were cultured in MRS medium containing antibiotics for 120 generations. Biomass was expressed as an absorbance value (A600).

## Results and discussions

*Characteristics of L. plantarum Lrld-22*

The strain Lrld-22 was isolated from Qinghai yak fermented milk, and subsequently domesticated with fermented *C. cathayensis* Sarg. and *T. grandis* milk. The domesticated strains were further screened from the fermented *C. cathayensis* Sarg. and *T. grandis* milk. Morphologically, the Lrld-22 was round with milky white colonies having smooth, raised surface, and neat, opaque edges. The colony diameter ranged from about 3 - 5 mm. The Gram-staining result was positive, and microscopy revealed bacillus (rod) shaped (Figure 1).



**(A)** **(B)**

**Figure 1.** Colony characteristics **(A)** and morphology **(B)** of *L. plantarum* Lrld-22.

*16S rRNA sequence analysis*

Bacterial universal primers were used to amplify a 1,470 bp DNA fragment of the 16S rRNA gene, and the sequence data were submitted to GenBank under the accession number SUB14284941. Phylogenetic analysis of the 16S rRNA sequence of strain Lrld-22 revealed more than 99% similarity to other *L. plantarum* strains, with 100% identity to strain DBNSCRS-2. Based on colony and morphological features, strain Lrld-22 was conclusively identified as *L. plantarum*.

*Genomic information of L. plantarum Lrld-22*

Data analysis and sequence assembly were performed on *L. plantarum* Lrld-22 sequencing sample, and yielded the genome assembly results and basic genome characteristics. The assembled sequences were spliced into a circular genome diagram (Figure 2). The results showed that the *L. plantarum* Lrld-22 genome sequence consisted of a single circular chromosome (Figure 2A) and two plasmids (Figures 2B and 2C). The whole genome length was 3,369,454 bp, with the circular chromosome accounting for 3,246,150 bp. The GC content (%) was 45.48%. The average gene length was 859.12 bp, with the longest measuring 313,638 bp, and the total read number was 3,284. *L. plantarum* is one of the largest species in the genome of lactobacilli, with typical genome lengths ranging from 3.2 to 3.4 Mbp (Kant *et al*., 2011; Stefanovic *et al*., 2017). However, there are certain differences in the characteristics of the *L. plantarum* genome of different strains, such as GC content, the length of the entire genome, the presence or absence of plasmids, the number of plasmids, *etc.* (Siezen *et al*., 2010; Yu *et al*., 2021).

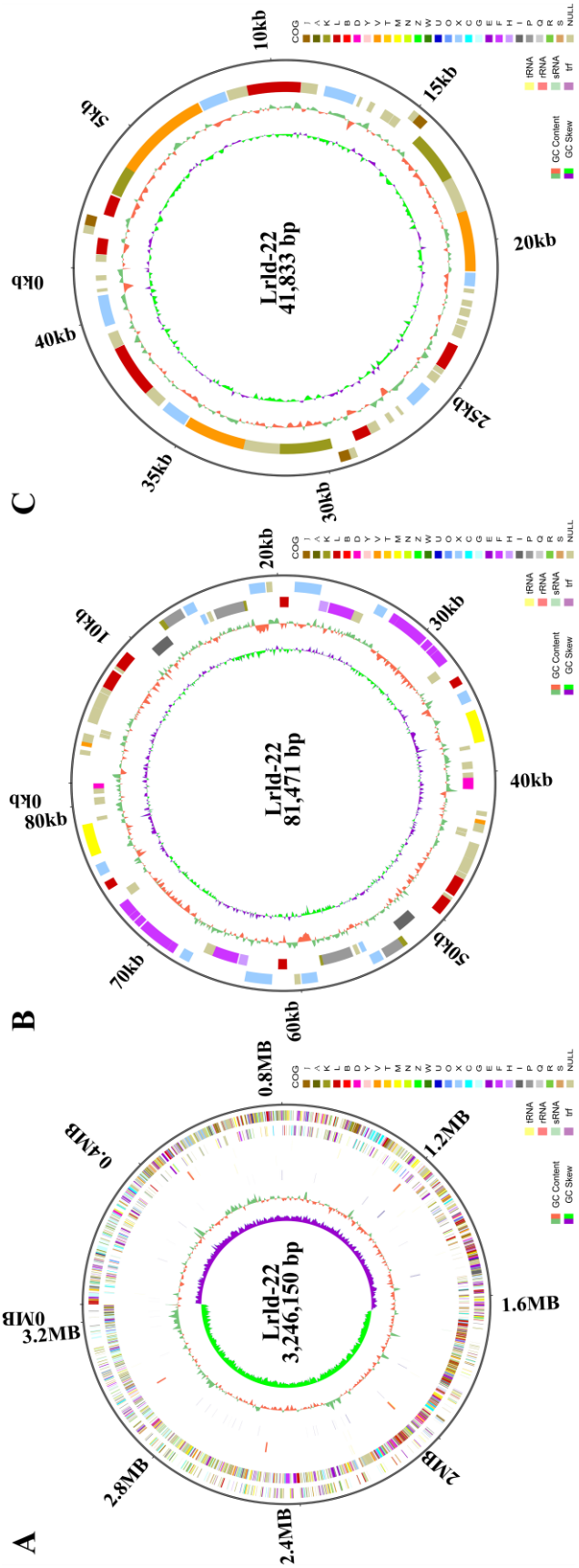



**Figure 2.** Genomic cycle **(A)** and plasmid cycle [**(B)** and **(C)**].

*Average nucleotide identity (ANI) analysis and evolutionary position*

ANI is a parallel sequence of the genome to determine whether a strain belongs to the same species or a subspecies. In contrast, TNI quantifies the nucleic acid matching ratio between genomes, providing a higher differentiation capacity for the genomic dataset (Sun *et al*., 2015). ANI can evaluate the degree of inter-genomic polymorphism, and determine the similarity between genomes. Generally, ANI value of 95% or higher indicates that the strains are the same (Richter and Rosselló-Móra, 2009). In our analysis, we found and grouped the ANI values for the Lrld-22 strains and the reference strains, including *L. acidophilus* YT1, *L. casei* FBL6, *L. paragasseri* JV-V03, *L. plantarum* Q180, and *L. reuteri* FN041. The ANI values of Lrld-22 and *L. plantarum* Q180 were 99.15%, confirming that the strain Lrld-22 was indeed *L. plantarum* (Richter and Rosselló-Móra, 2009) (Figure 3).

The phylogenetic tree is a fundamental visualisation tool in evolutionary biology, offering critical insights into evolutionary relationships, including inter-species affinities (Cardoni *et al*., 2022). The phylogenetic analysis showed that Lrld-22 and *L. plantarum* Q180 were more closely related. This suggested that the evolutionary relationship between the two strains was the newest, with minimal time required for genetic development.

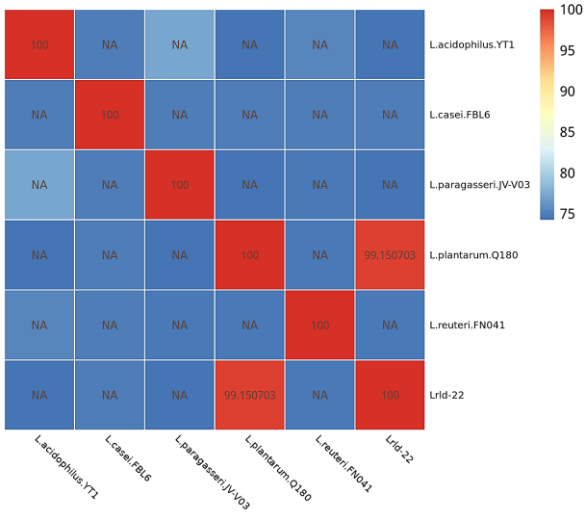



**Figure 3.** ANI heat map.

*Functional annotation of strain Lrld-22 genome*

*Orthologous groups of proteins (COGs) annotation of genome*

The COG database categorises protein functions into 26 categories, each of which consists of a straight-line homogenic sequence. By comparing the protein sequences and annotating it with a COG classification, the function of the sequence can be predicted (Liu *et al*., 2020). *L. plantarum* Q180, a well-characterised strain, serves as a valuable reference due to its strong bile salt tolerance and its ability to survive and colonise the human intestine (Al-Tawaha and Meng, 2018). Therefore, the genomic information of *L. plantarum* Q180 offers significant insights into understanding of *L. plantarum* Lrld-22. For *L. plantarum* Lrld-22, 2,653 genes (accounting for 80.79% of CDS) were annotated in the COG database. These gene annotations mainly involve three functional categories: cells, information transduction, and metabolism (Figure 4A). Specifically, 271 genes were associated with carbohydrate transport and metabolism, and 222 genes were related to amino acid transport and metabolism, indicating that *L. plantarum* Lrld-22 had strong ability to utilise carbohydrates and amino acids. Additionally, 152 gene annotations were obtained for cell wall, membrane, and envelope biogenesis, suggesting that the strain Lrld-22 had strong biofilm formation ability. Sixty-seven genes were associated with defence mechanisms, indicating some potential for environmental resistance. Other functional annotations included genes related to transcription, translation, ribosomal structure and biogenesis, post-translational modification, and protein turnover, suggesting that strain Lrld-22 possessed efficient protein synthesis and modification capabilities. Furthermore, 135 genes were involved in lipid transport and metabolism, while 116 genes were related to energy production and conversion. A total of 341 genes were categorised as either having unknown functions or being classified under "general function prediction only," indicating the need for further functional characterisation.

*Gene ontology (GO) analysis of the genome*

GO is used to comprehensively describe the features of genes and genetic products across living organisms, including their molecular functions, cellular locations, and the biological processes, in which they are involved (Peng *et al*., 2021). As shown in Figure 4B, GO analysis of *L. plantarum* Lrld-22 indicated that 6,044 genes were annotated, with biological processes, cell composition, and molecular


**Figure 4.** COG database annotation **(A)**, GO **(B)**, and KEGG **(C)** analysis for genome.

functions accounting for 49.14% (2,970), 12.92% (781), and 37.94% (2,293) of the total annotations, respectively. Within the biological processes category, the most prominent annotations included biological regulation (217 genes), cellular processes (1,044 genes), localisation (311 genes), metabolic processes (1,047 genes), regulation of biological processes (211 genes), and response to stimulus (79 genes). For the cellular component category, the annotations were primarily assigned to cellular anatomical entities (529 genes), intracellular (166 genes), and protein-containing complexes (86 genes). In the molecular functions category, the annotations were mainly distributed across four classifications: binding (785 genes), catalytic activity (1,111 genes), transcription regulator activity (100 genes), and transporter activity (220 genes). These findings provided a comprehensive overview of the functional and structural characteristics of the *L. plantarum* Lrld-22 genome.

*Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of genome*

The KEGG database integrates multiple data points to systematically analyse the functions and metabolic pathways of genetic expression products (Liu *et al*., 2020). In the KEGG annotation of *L. plantarum* Lrld-22, a total of 2,135 genes was found, as illustrated in Figure 4C. Most of the genes were annotations in the metabolic pathways of the four categories: metabolism (1,476 genes), environmental (249 genes), genetic (173 genes), and human diseases (107 genes). The main metabolic pathways were carbohydrate and amino acid metabolisms. Most of the genes were enriched in the metabolism of cofactors and vitamins, nucleotide metabolism,

energy metabolism, and lipid metabolism. Environmental annotation highlighted gene involvement in membrane transport and signal transduction. For genetic information processing, 173 genes were linked to pathways associated with protein folding, sorting and degradation, replication and repair, as well as translation and transcription pathways. Additionally, a few genes were annotated in pathways related to antimicrobial and antineoplastic; cancer, overview; cardiovascular disease; and infectious disease, bacterial.

Furthermore, 43 genes were involved in organ systems, including aging, the digestive system, the endocrine system, environmental adaptation, the immune system, and the nervous system. Of these, a significant portion was annotated in the endocrine system. The endocrine systems of *Lactobacillus* specifically manifest in blocking the infiltration of bacteria into the intestine, suppressing the bacteria, fighting infections, and increasing the diversity of microbial intestines (Neuman *et al*., 2015; Qi *et al*., 2021; Sabahi *et al*., 2023). Additionally, *Lactobacillus* activity within the endocrine system is linked to tumour suppression, enhanced immune response, improved digestion, vitamin and carbohydrate production, cholesterol reduction, toxin inhibition, and prevention of aging and radiation damage (Di Cerbo *et al*., 2015; Zhai and Chen, 2019; El-Sayed *et al*., 2021).

*Carbohydrate-active enzymes functional gene notation analysis*

The CAZy includes a family of enzymes that catalyse carbohydrate degradation, modification, and biosynthesis (Huang *et al*., 2021). CAZy contains five main enzyme families: glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), and auxiliary activities (AAs), along with carbohydrate-binding modules (CBMs). Carbohydrates play an important role in many biological functions, and analysing carbohydrate-related enzymes provides valuable insights into metabolic functions (Peng *et al*., 2021). In the genome of *L. plantarum* Lrld-22, the highest proportion of the CAZy annotation was for GH, with a percentage of 54% (Figure 5). GTs and CBMs followed, representing 29 and 14%, respectively. GH catalyses the hydrolysis of polysaccharides containing multiple 1,4-α-D-glucoside groups. This process provides a significant amount of energy for bacteria metabolic activities (Evanovich *et al*., 2019). Glycosyltransferases facilitate the formation of glycosidic bonds by transferring sugars to specific receptors like proteins, lipids, or other glycans, thus playing a vital role in polymer construction and supporting various biological functions (Bhat *et al*., 2019; Evanovich *et al*., 2019). CEs, which catalyse the de-esterification of various carbohydrate substrates, were also annotated at 2%, while auxiliary activity enzymes, a large class of redox-active enzymes that act on carbohydrates, were annotated at 1% (Bhat *et al*., 2019; Peng *et al*., 2021). These findings, along with the results of COG and CAZy, indicated that *L. plantarum* Lrld-22 had strong carbohydrate metabolism ability and good prebiotic potential. This was consistent with other strains of *L. plantarum*, which can extensively utilise a variety of sugar sources such as polysaccharides, oligosaccharides, and sugar alcohols to cope with complex environments (Liu *et al*., 2022; Hu *et al*., 2023). This metabolic ability is related to the CAZymes gene in the genome, which plays a key role in adapting to the intestinal environment, and regulating the function of intestinal epithelial cells (Peng *et al*., 2021; Liu *et al*., 2022; Hu *et al*., 2023).

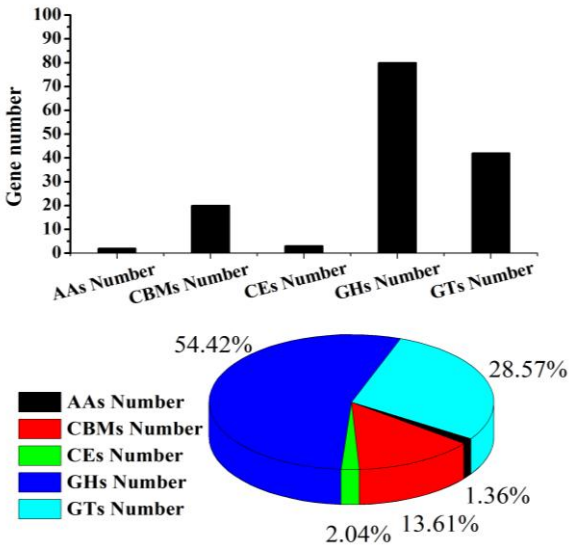



**Figure 5.** CAZy of *L. plantarum* Lrld-22.

*Functional analysis of fat metabolism genes*

Seven genes (Lrld-22GL000131, Lrld-22GL001496, Lrld-22GL002428, Lrld-22GL002653, Lrld-22GL002659, Lrld-22GL002660, and Lrld-22GL002667) were annotated as encoding 3-oxoacyl [acyl carrier protein] reductase, a key enzyme in the biosynthetic process of fatty acids, such as polyunsaturated fatty acids. Additionally, six genes (Lrld-22GL003134, Lrld-22GL000069, Lrld-

22GL001487, Lrld-22GL001746, Lrld-22GL002357, and Lrld-22GL002517) encode alcohol dehydrogenase, which is involved in fat catabolism. Furthermore, seven genes are involved in the decomposition and utilisation of glycerol: Lrld-22GL000315 (glycerol kinase), Lrld-22GL001087 (diacylglycerol kinase), Lrld-22GL001149 (glycerol-3-phosphate cytidylyltransferase), Lrld-22GL001195 (poly(glycerol-phosphate) alpha-glucosyltransferase), Lrld-22GL001525 (diacylglycerol kinase), Lrld-22GL001924 (diacylglycerol kinase), and Lrld-22GL002049 (CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase). Notably, three of these genes encode diacylglycerol glycerol kinase, which catalyses the transfer of a phosphate group from a high-energy donor molecule (such as ATP) to a specific target molecule (substrate), playing a key role in lipid metabolism. Additionally, Lrld-22GL000308, Lrld-22GL000589, and Lrld-22GL001498 were all annotated as acetyl-CoA carboxylase biotin carboxyl carrier protein, a complex consisting of three subunits, including biotin carboxylase, carboxyl transferase, and biotin carboxyl carrier protein, all of which are involved in the synthesis of fat. The genes Lrld-22GL000591, Lrld-22GL001501, Lrld-22GL000592, and Lrld-22GL001502 encode acetyl-CoA carboxylase carboxyl transferase subunit beta (accβ) and acetyl-CoA carboxylase carboxyl transferase subunit alpha (accA), respectively, which play an important role in the metabolism of fatty acids. accA, located in the cytoplasm, regulates fatty acid synthesis, while accβ, located in the mitochondria, is involved in fatty acid oxidation to regulate carnitine palm phthalein transferase. In addition, Lrld-22GL001495, Lrld-22GL001497, and Lrld-22GL001503 were annotated as [acyl-carrier-protein]: S-malonyltransferase, 3-oxoacyl-[acyl-carrier-protein] synthase II, and enoyl-[acyl-carrier protein] reductase I, respectively. These enzymes are involved in regulating bacterial fatty acid synthesis (Sabaitis and Powell, 1976; Yang *et al*., 2019).

*Safety assessment of L. plantarum Lrld-22*

*Antibiotic resistance*

Annotation of antibiotic resistance gene could help to identify specific drug resistance genes, and the corresponding antibiotics that confer resistance by blasting a comprehensive antibiotic resistance database (CARD, http://arpcard.mcmaster.ca). The database contains 13,293 genes, 377 types, 257 antibiotics, 124 phyla, and 3,369 species (Tang *et al*., 2023). According to Tang *et al*. (2023) and Wu *et al*. (2023), CARD serves as a valuable resource, referencing antibiotic resistance genes from different organisms, genomes, and polymers. These genes can be used to help researchers learn more about environmental, human, and animal bacterial resistance groups, and the mechanisms of antibiotic resistance. *L. plantarum* Lrld-22 annotated a total of nine antibiotic resistance genes from the CARD database. The results showed that these genes were associated with resistance to antibiotics, such as vancomycin, deoxycholate, phosphomycin, fluoroquinolone, and lincomycin, suggesting that *L. plantarum* Lrld-22 may have some resistance to these eight antibiotics. The potential mechanisms of vancomycin resistance in Lrld-22GL000031 may occur at the transcript level. VanG-type vancomycin resistance operon genes enable the synthesis of peptidoglycan with a modified C-terminal D-Ala-D-Aa to D-alanine-D-serine (Anisimova and Yarullina, 2019). The genes Lrld-22GL000112 and Lrld-22GL002949 may play a role in resistance to phosphomycin, mainly related to carbohydrate transport and metabolism (Mathur and Singh, 2005). The gene Lrld-22GL000112, part of the major facilitator superfamily transporter, acts as a multidrug resistance efflux pump. The possible resistance of the gene Lrld-22GL000764 to fluoroquinolone is also closely associated with the multidrug resistance efflux pump. Similarly, Lrld-22GL000955 and Lrld-22GL002944 are possibly involved in lincomycin resistance. The possible mechanism is to excrete lincomycin through the Macrolide-Lincosamide-Streptogramin B efflux pump (Mathur and Singh, 2005; Rozman *et al*., 2023). The genes Lrld-22GL002795 and Lrld-22GL002796 may be resistant through the VanC and VanG type vancomycin resistance operon, respectively, which are related to regulating peptidoglycan synthesised with modified C-terminal D-Ala-D-Aa to D-alanine-D-serine. Additionally, the gene Lrld-22GL002960 may be resistant to bacitracin through undecaprenyl pyrophosphate phosphatase, which plays a role in lipid transport and metabolism. Despite these annotations, experimental test results showed that *L. plantarum* Lrld-22 did not exhibit resistance to the antibiotics. In comparison, *L. paracasei* annotated have been shown to carry multiple antibiotic resistances genes (Rozman *et al*., 2023). The six

resistant genes *arlR*, *arlS*, *patB*, *gyrA*, *gyrB*, and *efmA* are associated with resistance to fluoroquinolone antibiotics, with some involved in regulating antibiotic excretion (Mathur and Singh, 2005; Rozman *et al*., 2023). Moreover, Devirgiliis *et al*. (2009) identified the antibiotic-resistant gene *tetM* in the transistor *Tn916* of *L. paracasei*, while Tn 916 also detected the *tetM* gene in *Lactococcus garvieae* and *Lactococcus lactis* (Mathur and Singh, 2005; Devirgiliis *et al*., 2009; Rozman *et al*., 2023). The *rpsL* gene, which encodes the nucleosaccharide protein S12, has mutations that affect the advanced structure of 16S rRNA to resist streptomycin (Mathur and Singh, 2005; Rozman *et al*., 2023).

*Virulence factor*

Virulence factors mainly include bacterial toxins, bacterial adhesion cell surface proteins, cell surface carbohydrates, and bacterial pathogenic hydrolases, which promote microbial self-infection, and cause specific host diseases (Chen *et al*., 2005; Wu *et al*., 2023). In *L. plantarum* Lrld-22, a total of 107 genes were annotated as potential virulence factors, of which 14 were clearly confirmed, with ten having a score above 60. Some of these were the bacterial adhesive protein, the capsular polysaccharide synthesis enzyme Cap8J, the acyl carrier protein, and the ATP-dependent protease. Two adhesion-related genes, Lrld-22GL000274 and Lrld-22GL003134, were discovered in *L. plantarum* Lrld-22, and homogeneous gene comparisons showed similarities with adhesion genes in *Listeria monocytogenes* SLCC7179 and *Listeria innocua* Clip11262 (Tang *et al*., 2023; Wu *et al*., 2023). Although these genes are identified as toxic factors in the toxicity factor database due to their involvement in the adaptation, survival, or attachment of pathogenic bacteria in host environments, they are not inherently pathogenic. In the absence of other virulence mechanisms, such genes may be beneficial, enhancing bacterial adaptability and promoting cell viability. In addition, the toxicity factor chromosome III (Lrld-22GL002878) has been detected in these genes, but this gene is also found in commercial probiotics, such as the generally regarded as safe probiotic strain *L. plantarum* 299V, widely used in China, and the commercial *L. plantarum* JDM1, which is not blood-soluble (Hu *et al*., 2023; Wu *et al*., 2023). Lrld-22 was also not blood-soluble, further suggesting its probiotic potential.

*Stress-related genes of L. plantarum Lrld-22*

The survival of probiotics in the gut is one of the important indicators of probiotics, with low pH and bile salts stress probiotics. Bile salts not only facilitate fat absorption but also act as surfactants, damaging the integrity of cell membranes, and producing free radicals that lower intracellular pH (Al-Tawaha and Meng, 2018). *L. plantarum* AR113 has been shown to have good gastrointestinal viability, largely due to the gene encoding bile saline hydrolysase, which contributes to bile tolerance (Wang *et al*., 2021). This strain also has cholesterol-clearing ability, which helps to regulate cholesterol intake in patients with cardiovascular diseases (Sabahi *et al*., 2023). In the genome of strain *L. plantarum* Lrld-22, the gene Lrld-22GL002562 was annotated as an MFS-type transporter of the Atg22 family involved in bile tolerance. In addition, the gene Lrld-22GL001987, annotated as broad-spectrum tolerance protein 13, may enhance the strain Lrld-22's resistance to various environmental stresses, including temperature fluctuations, salt stress, ethanol stress, glucose starvation, and oxidation stress. Other genes, such as Lrld-22GL002846 and Lrld-22GL000075, act as transcription factors and bind to DNA to regulate peroxidation and oxidative stress, respectively. Meanwhile, catalase, thioyl peroxidase, and glutathione peroxidase-related genes have positive effects on oxidative stress. The gene Lrld-22GL000975, annotated as a transcriptional regulator, likely governs the stress and heat shock responses of *L. plantarum* Lrld-22, contributing to its adaptation to environmental temperature changes.

During industrial production, probiotics may encounter temperature stress. High temperatures can induce the expression of conserved heat shock proteins, such as GroES, GroEL, GrpE, DnaK, and DnaJ, which protect against thermal damage (Wu *et al*., 2023). Cold shock protein-related genes, belong to the CSP family, have been found in many microorganisms, and are associated with bacterial adaptation and survival under low-temperatures conditions (Fiocco *et al*., 2007).

## Conclusion

The entire genome of a strain of *L. plantarum* Lrld-22 was sequenced, and its genome function was annotated. The genome size of strain Lrld-22 was

3.246 Mbp, with two plasmid sequences of 81,471 and 41,833 bp, respectively. Functional analysis revealed that *L. plantarum* Lrld-22 exhibited efficient carbohydrate utilisation and fatty acid metabolism. Additionally, its genomic characteristics suggested strong safety and promising potential for various applications.

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