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Comparative genomic analysis of *Lactococcus lactis* isolates from Chinese traditional cheeses reveals genomic diversity and functional adaptation

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ABSTRACT

Lactococcus lactis, a major starter culture in the dairy industry, has been widely applied in food fermentation. While current research has primarily focused on evaluating its role during fermentation, genomic investigations into its genetic diversity and functional adaptability remain limited. In this study, 199 *L. lactis* strains isolated from Chinese traditional artisanal cheeses (72 bovine, 71 goat, and 56 yak milk cheese isolates) were subjected to comparative genomic analysis. Genomic characteristic analysis indicated that bovine milk strains possess larger genomes and the highest number of unique genes. Functional characterization further demonstrated notable differences in carbohydrate metabolism among strains from different sources, with yak milk strains enriched in enzymes involved in complex polysaccharide degradation, including members of the carbohydrate esterases family. Moreover, strains from different sources exhibit distinct strategies for lactose hydrolysis and metabolic utilization, reflecting adaptive evolution to their specific nutritional niches. Analysis of the antibiotic resistance profile suggests that *L. lactis* predominantly harbors glycopeptide and lincosamide resistance genes, encompassing four distinct resistance mechanisms. Collectively, this study reveals the genetic diversity and adaptive evolution of *L. lactis* strains from different sources and identifies key genes associated with carbohydrate degradation, lactose metabolism, and antibiotic resistance, providing concrete genetic evidence for the selection of efficient and safe industrial fermentation strains.

1. Introduction

Lactococcus lactis is a key model species of lactic acid bacteria, which has been isolated from a variety of habitats, including the animal gut[1], soil[2], dairy products[3], and plants[4]. Historically, *L. lactis* was divided into 2 lineages[5], one comprising *L. lactis* subsp. *lactis* and the other *L. lactis* subsp. *cremoris*, with the latter recently reclassified as the separate species *L. cremoris*[6]. As a principal member of industrial starter cultures, *L. lactis* has long been employed in the food industry and is recognized as safe under the generally recognized as safe (GRAS) designation[7]. It is primarily utilized in the fermentation of dairy products, including yogurt, cheese, and butter, owing to its ability to efficiently metabolize lactose and rapidly produce lactic acid[8-9]. However, in response to the evolving challenges of the food market, where products are increasingly diversified in flavor, texture, and health benefits, more efficient strategies are required to develop and utilize the natural biodiversity of starter culture strains[10]. Understanding the genetic basis of functional traits in *L. lactis* not only advances knowledge of the mechanisms underlying industrially relevant phenotypes but also supports the targeted screening of starter cultures with desirable properties.

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Although the current applications of *L. lactis* in fermented foods are largely associated with dairy fermentations, evidence suggests that these microorganisms originated from plant-associated niches, and they are now regarded as domesticated strains in contrast to their so-called wild counterparts[11]. Passerini et al.[12] demonstrated that environmental isolates represent the ancestral forms, whereas domesticated strains emerged through founder events that conferred a growth advantage in dairy environments. Specifically, these domesticated strains acquired plasmids involved in lactose and casein metabolism, thereby facilitating their adaptation to the dairy niche[13]. These findings highlight a close association between the genetic features of *L. lactis* and its ecological environments. A similar pattern has been reported in other lactic acid bacteria, such as *Leuconostoc mesenteroides*, in which strains from dairy and plant origins exhibit differences in galactose metabolism[14].

Chinese traditional artisanal cheeses provide a unique context for investigating the genetic adaptation of *L. lactis*. These cheeses are produced by ethnic minority groups using various milk sources, including bovine milk, goat milk, and yak milk[15-17]. Compared with industrially produced European cheeses, these products are made without the addition of commercial starter cultures and instead rely primarily on back-slopping, a process in which residual fermentation products from a previous batch are inoculated into fresh substrate[18]. This practice allows resident microorganisms to maintain long-term, continuous propagation across different fermentation substrates, thereby promoting selective adaptation and genetic evolution of the strains[16,19]. Notably, the nutritional composition of different milk sources exhibits significant variation. Compared with bovine milk, goat and yak milk contain a greater diversity and abundance of milk oligosaccharides, and differences are also observed in amino acid profiles and lipid composition[20-22]. Considering these substrate differences, we hypothesize that *L. lactis*, as a key microbial species in cheese fermentation, may display genomic diversity across cheeses produced from different milk sources.

Comparative genomics has emerged as a powerful approach for elucidating population evolutionary patterns among strains from distinct ecological niches, offering new perspectives on the genetic bases of habitat adaptation, metabolic diversity, and antimicrobial resistance[23-25]. Multiple comparative genomic studies have revealed that, even within a single species, strains isolated from different sources exhibit extensive genetic diversity, which is closely associated with their respective ecological niches, including *Lentilactobacillus kefiranofaciens*[26], *Limosilactobacillus reuteri*[27], and *Lacticaseibacillus casei*[28]. Although a few studies have reported the intraspecific diversity of *L. lactis*, these investigations have mainly focused on comparisons between dairy-derived and non-dairy-derived strains. Li et al.[29] demonstrated that dairy isolates of *L. lactis* exhibit higher copy numbers in glycosyltransferases (GTs) families, whereas plant-derived isolates are enriched in glycoside hydrolases (GHs) families. Similar study has also reported that dairy-derived *L. lactis* strains harbor more acid-producing and lactose metabolism genes than those isolated from environmental source[18]. As highlighted earlier, previous studies have indicated that there are significant differences in the nutritional composition of milk across various sources. However, the relationship between these nutritional variations and the genomic characteristics of dairy-derived *L. lactis* remains unclear, thereby limiting our understanding of strain adaptation to nutritional environments and their genetic variation.

In this study, we performed whole-genome sequencing on 199 *L. lactis* strains isolated from Chinese traditional artisanal cheeses made with different milk sources. Pangenome analysis revealed their genetic diversity, and functional genomic profiling identified genes differentially enriched among strains, providing insights into their adaptation to distinct nutritional environments. Furthermore, considering the safety of *L. lactis* in food applications, we investigated the antibiotic resistance genes (ARGs) of these strains. This study offers novel perspectives on the genomic diversity of *L. lactis* and contributes to their development and application in the food industry.

2. Materials and methods

2.1 Strain isolation and whole genome sequencing

Chinese traditional artisanal cheeses were collected from Xinjiang, Inner Mongolia, Yunnan, Tibet, and other regions of China, including Rushan, Naidoufu, and Naigeda made from bovine milk, Rubing from goat milk, and Qula from yak milk. A total of 199 *L. lactis* strains were isolated from these fermented cheeses. Specifically, cheese samples were serially diluted to appropriate gradients and spread onto de Man, Rogosa and Sharpe medium (MRS) agar plates, which were then incubated in an inverted position at 37 ℃ for 48 h. Colonies exhibiting typical lactic acid bacteria morphology were selected for identification and further purified on MRS agar plates. Purified strains confirmed as *L. lactis* were grown in MRS broth under aerobic conditions at 30 ℃. Genomic DNA was subsequently extracted from the cultures and sequenced by Novogene using the Illumina NovaSeq platform with 150-bp paired-end reads.

2.2 Isolates assembly and quality control

Genomes of 199 genomic samples were assembled with MEGAHIT (v1.2.9) using default k-mer parameters, retaining contigs ≥ 200 bp. Assembly quality was evaluated using the 'lineage_wf' workflow of CheckM (v1.1.3), and only assemblies with completeness ≥ 90% and contamination ≤ 5% were retained.

2.3 Core/Pan-genome and phylogenetic analyses

Functional genes of each strain were predicted using Prokka (v1.14.6) with default parameters, and core and pan-genomes across strains were identified using Roary (v3.13.0) based on the annotated gene files. Taxonomic classification and phylogenetic analysis were performed using GTDB-Tk, and maximum-likelihood trees based on universal marker gene sets were visualized with iTOL.

2.4 Genome functional annotation and classification

Functional annotation was performed using eggNOG-mapper (v2.1), which utilizes the eggNOG database, a comprehensive resource of orthologous groups and functional annotations covering a wide range of taxa. This tool assigns functional categories, including Clusters of Orthologous Groups of proteins (COG) classifications, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and Gene Ontology (GO) terms, through fast orthology mapping. In this study, protein-coding genes from each genome were annotated with eggNOG-mapper using default parameters to obtain functional information and categorize genes across strains.

2.5 CAZymes analysis

Carbohydrate-active enzymes (CAZymes) were annotated using the dbCAN2 meta server. Briefly, protein sequences predicted from each genome were obtained from Prokka annotation and subjected to the run_dbcan pipeline (v4.1.4) with default settings. The identified CAZymes were then categorized into GHs, GTs, carbohydrate esterases (CEs), polysaccharide lyases (PLs), auxiliary activities (AAs), and carbohydrate-binding modules (CBMs).

2.6 Single nucleotide polymorphisms (SNPs) analysis

SNPs were identified using a pan-genome reference-based workflow. Roary (v3.13.0) was run on the annotated genomes to generate the pan-genome reference. For each isolate, Snippy (v4.6.0) mapped quality-controlled reads to this reference and called variants with default parameters. Per-sample results were combined with snippy-core to produce a multi-strain SNPs alignment for downstream analyses.

2.7 ARGs analysis

ARGs were identified using the Resistance Gene Identifier (RGI, v6.0.5) against the Comprehensive Antibiotic Resistance Database (CARD, v4.0.1). Predicted protein sequences from each genome were submitted to RGI with default parameters, and gene annotations were assigned based on the best hits to the CARD reference. The resulting annotations were further categorized by drug class and resistance mechanism according to the CARD ontology.

2.8 Statistical analysis

Statistical analyses were conducted using R (v4.4.0). The Wilcoxon rank-sum test or Fisher's exact test was applied for pairwise comparisons, while the Kruskal-Wallis test was employed for comparisons involving multiple groups. The Benjamini-Hochberg procedure was applied to control the false discovery rates (FDR).

3. Results

3.1 Genomic features of *L. lactis* isolates from different dairy sources

A total of 199 *L. lactis* strains were isolated from Chinese traditional cheeses. Based on the type of milk used for cheese production, the strains were categorized as 72 bovine milk isolates, 71 goat milk isolates, and 56 yak milk isolates. Genome sizes varied among strains, with bovine, goat, and yak milk isolates averaging (2.27 ± 0.14), (2.20 ± 0.69), and (2.26 ± 0.79) Mbp, respectively (Fig. 1A). Notably, goat milk strains exhibited significantly smaller genomes compared with bovine and yak milk strains ($P < 0.001$). To investigate the genetic diversity and functional differences among strains of different origins, coding genes were predicted from the genomes of the isolated strains, and the pan- and core-genome sets of

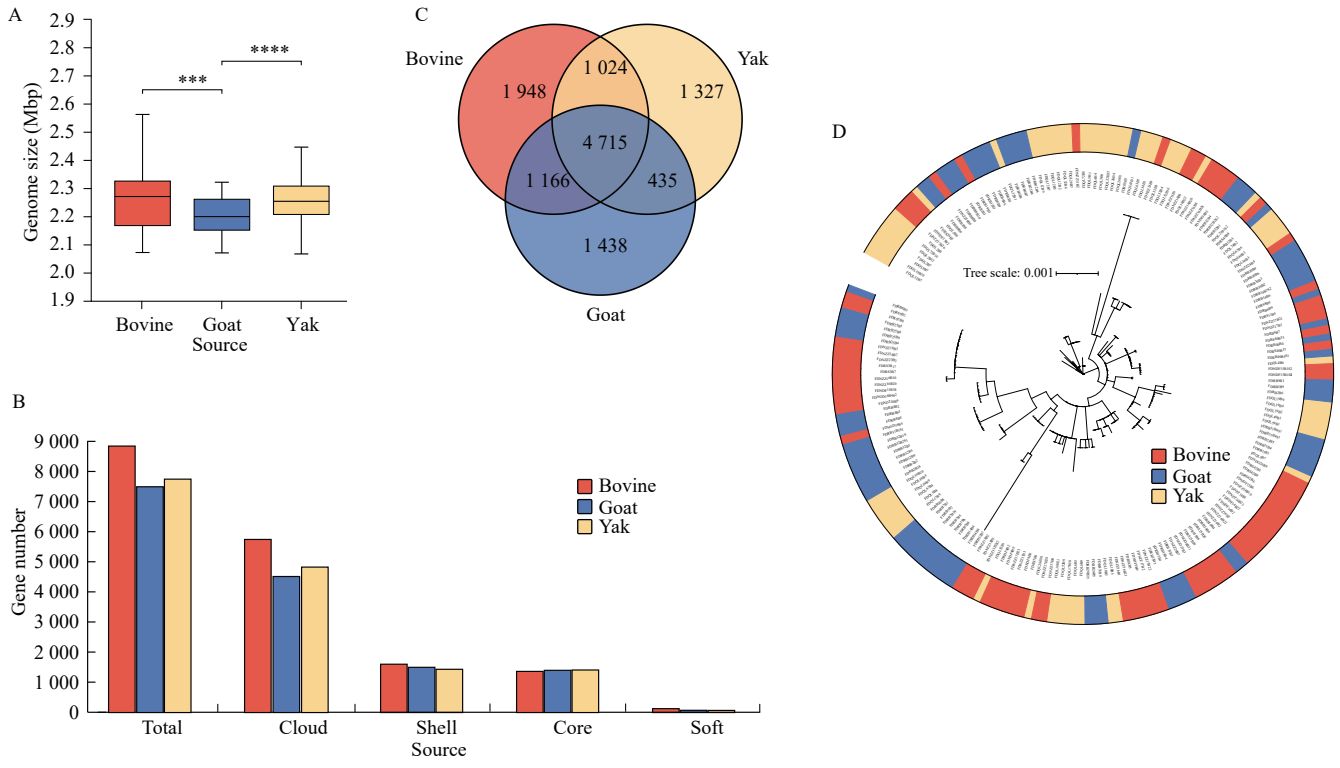

Fig. 1 Genomic characterization and evolutionary relationship of 199 *L. lactis* strains. (A) Genome size. (B) Comparison of pan-genomes and core genes among *L. lactis* isolates from different sources. (C) Unique and shared genes among *L. lactis* isolates from different sources. (D) A phylogenetic tree was built for 199 *L. lactis* genomes based on a concatenated alignment of 120 universally distributed bacterial single-copy genes. Data are presented as mean ± standard deviation (SD), with *** and **** indicating statistical significance at $P < 0.001$ and $P < 0.000\ 1$, respectively.

199 *L. lactis* isolates were constructed. Comparative analysis of the pan-core genomes revealed that the number of core genes (core and soft gene) did not differ significantly among the three groups, whereas strains isolated from bovine and yak milk harbored more accessory genes (cloud and shell gene) than those from goat milk (Fig. 1B). Furthermore, all three groups shared 4 715 genes, and the bovine milk strains contained the largest number of unique genes (Fig. 1C). In addition, we constructed a phylogenetic tree of 199 *L. lactis* isolates to evaluate the evolutionary relationships among them. The results showed that strains from different sources were dispersed across various branches, with no evident clustering according to their origin (Fig. 1D).

3.2 Functional annotation analysis of *L. lactis*

The COG annotation of *L. lactis* revealed that these isolates encompass a wide range of functional categories, including cellular processes and signaling, information storage and processing, metabolism, and poorly characterized functions (Fig. 2A). Comparative analysis of functional category distributions revealed that category replication, recombination and repair (category L) represented the largest proportion in isolates obtained from bovine and yak milk, whereas transcription (category K) was predominant in isolates derived from goat milk. We further performed pairwise comparisons of all annotated functional categories among the bovine, goat, and yak groups. The results showed that within the broad category of Metabolism, significant differences were detected only in lipid transport and metabolism (category I), where isolates from bovine milk exhibited significantly higher counts than those from both yak- and goat-derived milk (Fig. 2B, $P < 0.000\ 1$). Within the information storage and processing category, yak-derived isolates contained significantly more genes in category K than goat-derived isolates (Fig. 2C, $P < 0.001$). In category L, bovine-derived isolates harbored significantly higher counts than goat-derived isolates ($P < 0.01$), whereas no significant difference was detected relative to yak-derived isolates. Additionally, bovine-derived isolates showed significantly higher functional representation in most categories of cellular processes and signaling class (Fig. 2D), such as defense mechanisms (category V) and intracellular trafficking, secretion, and vesicular transport (category U).

In industrial applications, *L. lactis* is commonly employed as a starter culture for food fermentation, contributing to product quality primarily through the metabolic production of flavor compounds. To further explore potential functional differences, we analyzed COGs associated with category I. A total of 6 differential COGs were identified among the 3 groups of strains from different sources (Fig. 2E), namely COG0623, COG0657, COG1182, COG1211, COG1502, and COG1835. Comparative analysis revealed that bovine milk strains carried significantly higher copy numbers of

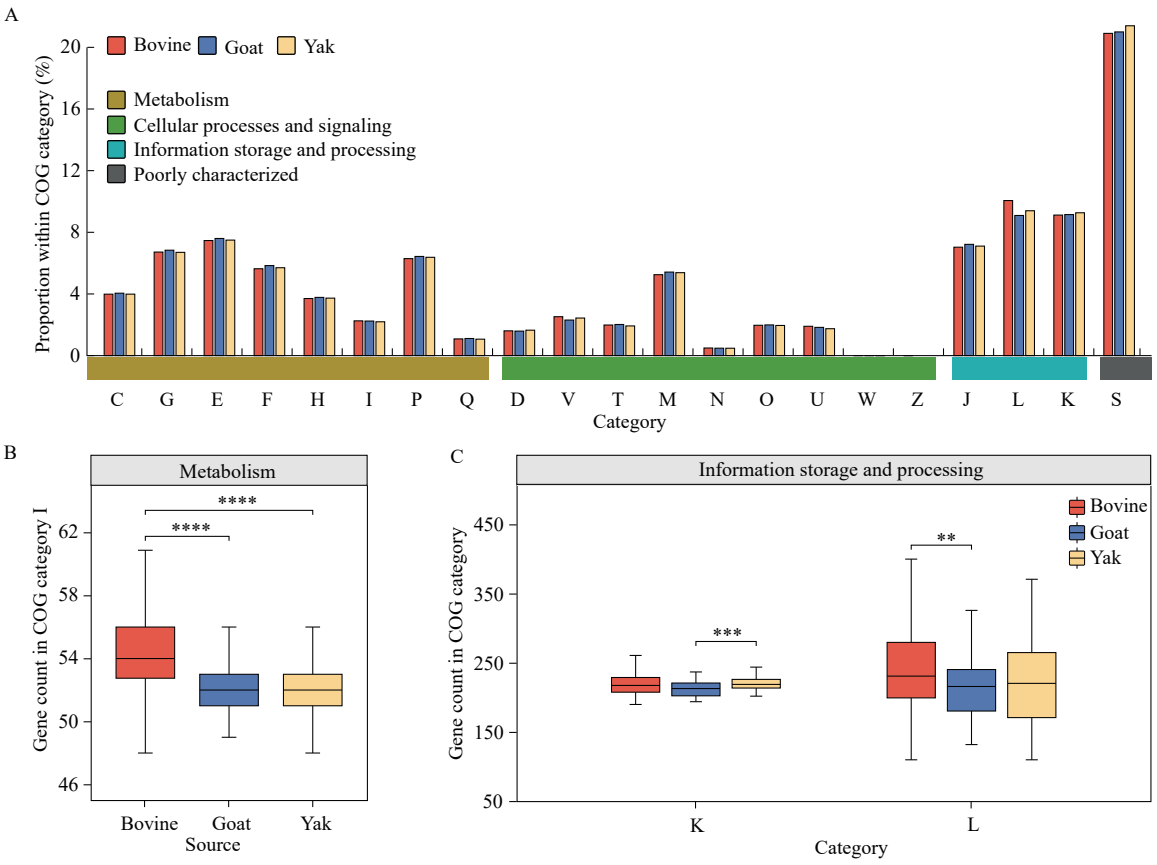

Fig. 2 Distribution of functional categories in COG annotations. (A) Comparison of COG category proportions among *L. lactis* isolates from different sources. (B-D) Differences in gene counts across strain sources within metabolic categories (B), information storage and processing categories (C), and cellular processes and signaling categories (D). (E) Differences in COGs copy numbers among strains from different sources. Subscript 1-6. COG0623, COG0657, COG1182, COG1211, COG1502, and COG1835. Data are presented as mean ± SD, with *, **, ***, and **** indicating statistical significance at $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.000\ 1$, respectively.

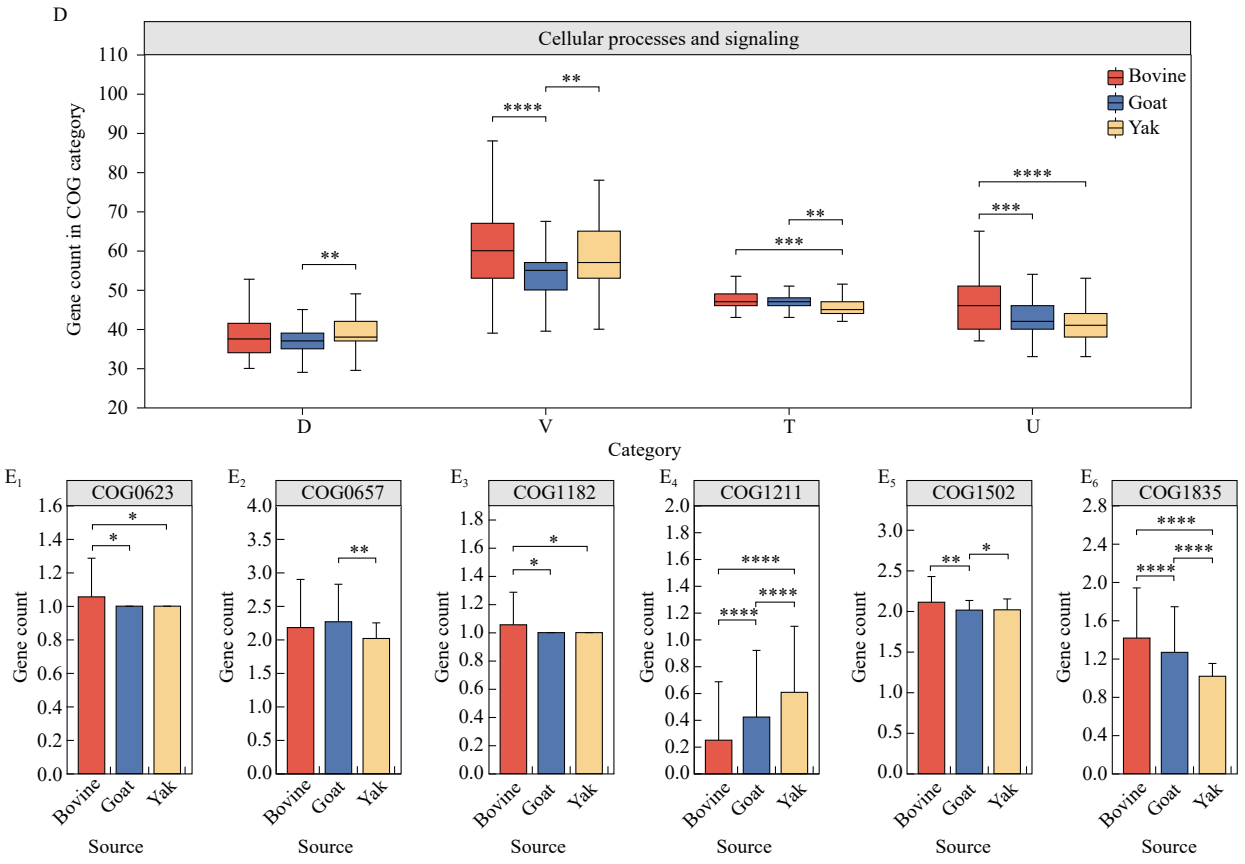

Fig. 2 (*Continued*)

COG0623, COG1182, and COG1835 than strains isolated from goat milk and yak milk ($P < 0.05, P < 0.000\ 1$). In contrast, the copy number of COG1211 was significantly enriched in yak milk strains compared with the other 2 groups ($P < 0.000\ 1$). Overall, these findings demonstrate that isolates from different sources differ significantly in their genomic functional profiles.

3.3 KEGG pathway analysis of *L. lactis*

KEGG functional annotation of 199 *L. lactis* strains identified a total of 1 828 KEGG orthology (KOs). Kruskal-Wallis testing subsequently revealed 282 KOs with significant differences. Enrichment analysis of these differential KOs highlighted the top 10 pathways (Fig. 3A), many of which were associated with metabolic processes, particularly those classified under categories riboflavin metabolism, porphyrin and chlorophyll metabolism, galactose metabolism and thiamine metabolism. Pairwise comparisons among the 3 isolation sources revealed 175 differential KOs between bovine and goat milk strains, with porphyrin metabolism showing the strongest enrichment (Fig. 3B). In comparison with strains derived from bovine or goat milk, the yak milk strains exhibited significant enrichment of differential KOs, with pentose and glucuronate interconversions emerging as the most prominent pathway, alongside teichoic acid biosynthesis and β-lactam resistance pathways (Figs. 3C, D). These results underscore marked functional divergence among strains from distinct ecological niches, which may be influenced by their adaptability to fermentation substrates.

3.4 Carbohydrate analysis of *L. lactis*

Given the substantial differences in the composition and abundance of carbohydrates among various milk sources, we predicted CAZymes to investigate how *L. lactis* strains adapt to the carbon source environments of specific milks. Analysis of the 199 genomes revealed a total of 71 distinct CAZymes families, including 35 GHs, 21 GTs, 8 CBMs, 5 CEs, 1 PLs, and 1 AAs. The number of genes encoding CAZymes varied markedly among the analyzed strains, ranging from 131 to 189, indicating considerable strain-specific diversity in carbohydrate-active potential. GHs were the most abundant CAZymes genes across all strains, followed by GTs, highlighting the central role of glycosidic bond hydrolysis and synthesis in *L. lactis* (Fig. 4A). Principal coordinate analysis (PCoA) derived from CAZymes gene counts demonstrated that strains from yak, bovine, and goat milk sources formed distinct clusters, with PCo1 and PCo2 accounting for 22.02% and 13.24% of the total variance, respectively (Fig. 4B). To investigate the carbohydrate utilization potential of *L. lactis* strains from different milk sources, we statistically analyzed the number of genes encoding various CAZymes families. Yak milk strains exhibited a significantly higher number of GHs genes compared with goat milk strains ($P < 0.01$), but showed no significant difference relative to bovine milk-derived strains (Fig. 4C). Notably, CEs family genes were significantly enriched in yak milk strains compared with both bovine and goat milk strains (Fig. 4D, $P < 0.000\ 1$), suggesting enhanced capacity for carbohydrate ester hydrolysis in these strains.

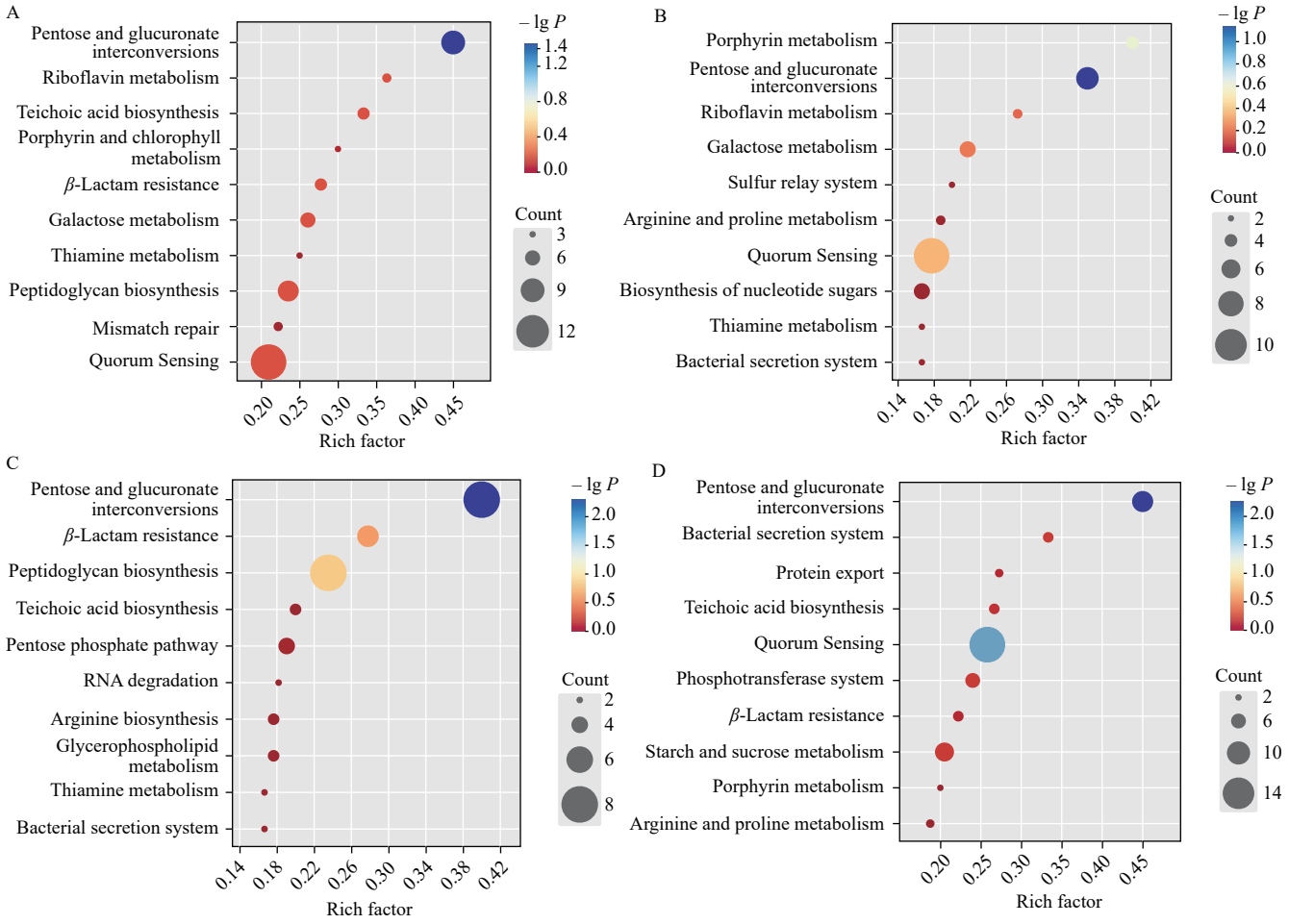

Fig. 3 KEGG pathway enrichment analysis. (A) Top 10 KEGG pathways enriched with differential KOs among *L. lactis* isolates from different sources. (B-D) Top 10 KEGG pathways enriched with differential KOs between bovine and goat milk isolates (B), bovine and yak milk isolates (C), and goat and yak milk isolates (D).

Lactose hydrolysis is a key physiological process during cheese production. Since enzymes responsible for lactose breakdown are primarily classified in the GH1 and GH2 families, we examined the abundance of these CAZymes families across *L. lactis* strains from different sources. Yak milk-derived strains contained the fewest GH1 genes while showing a significant increase in GH2 gene abundance (Figs. 4E and F). Moreover, these strains were significantly enriched in GH4, GH8, GH67, and CE20 compared with strains from other sources (Fig. 4G). Together, these results reveal pronounced differences in the CAZymes profiles of yak milk-derived strains, potentially reflecting adaptation to the higher diversity and abundance of oligosaccharides in yak milk.

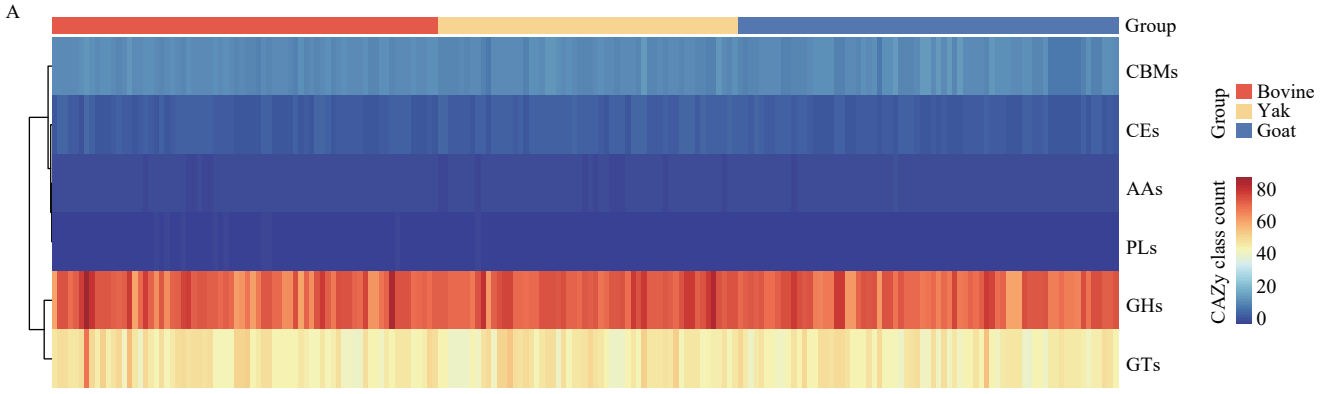

Fig. 4 Carbohydrate analysis of *L. lactis* strains. (A) Heatmap showing the distribution of CAZymes annotated in 199 *L. lactis* strains. (B) PCoA showing clustering patterns based on the abundance of CAZymes genes in *L. lactis* strains, according to strain origin. (C-F) Differences in gene counts of GHs (C), CEs (D), GH1 (E), and GH2 (F) among *L. lactis* isolates from different sources. (G) Distribution of genes encoding specific carbohydrate utilization enzymes among *L. lactis* isolates from different sources. Data are presented as mean ± SD, with *, **, and **** indicating statistical significance at $P < 0.05$, $P < 0.01$ and $P < 0.000\ 1$, respectively.

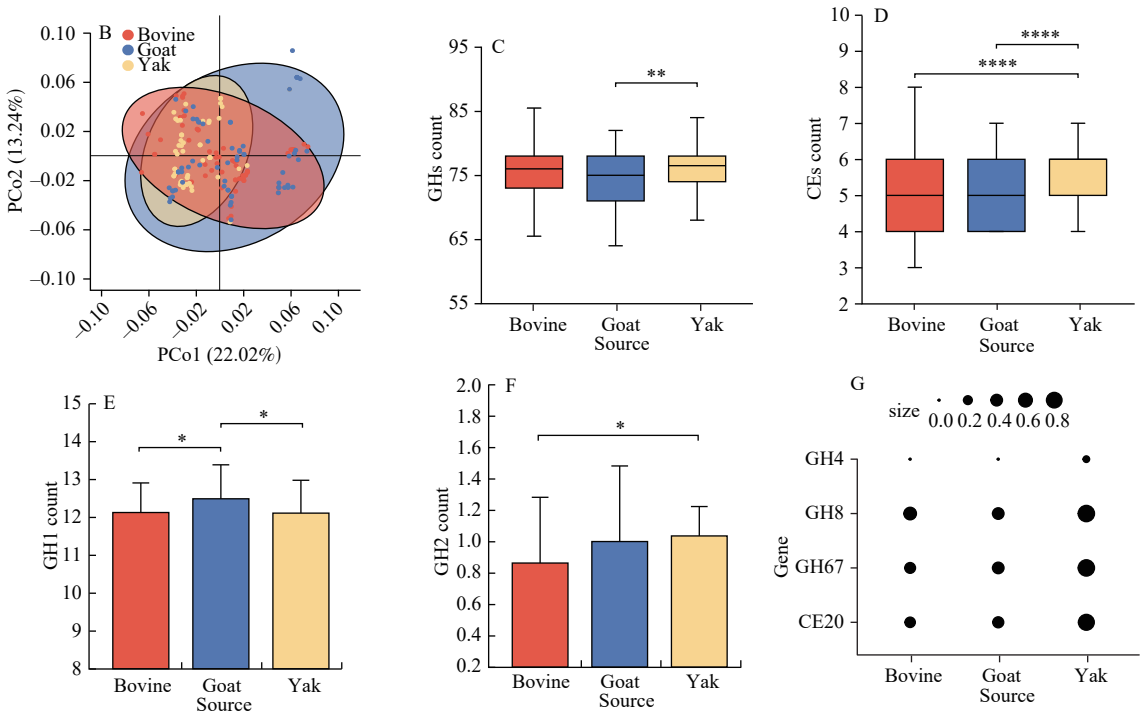

Fig. 4 (*Continued*)

3.5 *SNP analysis of L. lactis*

SNPs analysis of 199 *L. lactis* strains identified 67 643 variant sites. PCoA based on these SNPs did not reveal clear separation among strains from different sources (Fig. 5A). Nevertheless, yak milk-derived strains formed a more compact cluster than those from bovine and goat milk, suggesting reduced genetic diversity within the yak milk population. Based on the functional differences observed

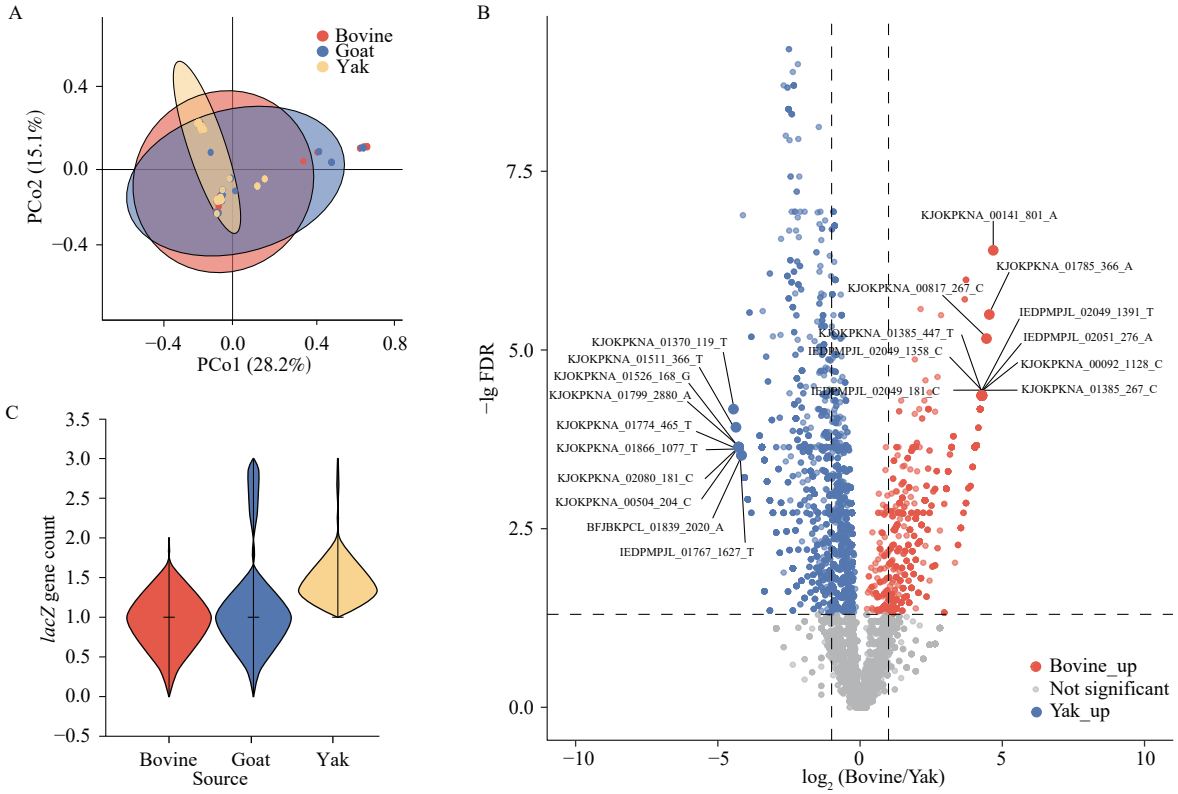

Fig. 5 SNPs analysis of *L. lactis* strains. (A) PCoA plot showing clustering patterns based on SNPs-level variation in *L. lactis* strains according to strain origin. (B) Volcano plot showing the differential SNPs between bovine and yak milk isolates. Red and blue dots indicate SNPs that are upregulated in the bovine and yak group, respectively. (C) Differences in *lacZ* gene counts among *L. lactis* isolates from different sources.

above between strains isolated from yak milk and those from bovine milk, we further investigated their genetic divergence at the SNPs level. Fisher's exact test identified 7 491 significantly differentiated SNPs between the 2 groups, of which 5 438 were significantly enriched in yak milk-derived strains (Fig. 5B). Subsequent analysis focused on the top 10 SNPs enriched in each group (Table S1). Gene annotation of the SNPs revealed that several mutations associated with carbohydrate metabolism, including KJOKPKNA_01511_366_T (hexokinase, EC 2.7.1.21) and IEDPMPJL_01767_1627_T (*β*-galactosidase, EC 3.2.1.23), were preferentially enriched in yak milk-derived strains, underscoring genetic variation that may contribute to their distinct metabolic potential. Previous studies have shown *β*-galactosidase plays a crucial role in milk fermentation by catalyzing the hydrolysis of lactose into glucose and galactose[30]. Protein alignment revealed that a nucleotide substitution at position 1 627 in the *β*-galactosidase gene resulted in an amino acid change from alanine to valine, potentially altering enzyme activity and stability. Examination of *lacZ* copy number across strains showed higher counts in yak milk strains compared with other sources, though the difference was not statistically significant (Fig. 5C). These results indicate that yak milk strains may harbor distinct genetic variations in *β*-galactosidase, which could contribute to differences in lactose metabolism among strains.

3.6 ARGs analysis of *L. lactis*

ARGs provides insight into the potential safety risks of bacterial strains and their capacity to survive under selective pressures, which is particularly important when evaluating strains for food fermentation or probiotic applications. ARGs were predicted in the strains using the CARD database. Across 199 *L. lactis* strains, a total of 486 ARGs were identified, with the number of genes per strain ranging from 1 to 8. These ARGs were categorized into 11 drug classes, among which classes glycopeptide antibiotic and lincosamide antibiotic were broadly distributed across all strains (Fig. 6A). In contrast, class streptogramin A antibiotic, streptogramin B antibiotic and macrolide antibiotic were detected exclusively in yak milk- and goat milk-derived strains, class phenicol antibiotic were absent in yak milk-derived strains. Notably, class pleuromutilin antibiotic were found only in bovine milk-derived strains. Yak milk-derived strains carried a significantly higher number of ARGs compared with bovine milk-derived strains ($P < 0.05$, Fig. 6B). Analysis of the resistance mechanisms encoded by these genes identified 4 types, including antibiotic target alteration, antibiotic efflux, antibiotic target protection and antibiotic inactivation (Fig. 6C). All 4 mechanisms were present across strains from different sources, with antibiotic target alteration and antibiotic efflux representing the predominant types. Notably, the antibiotic inactivation resistance mechanism

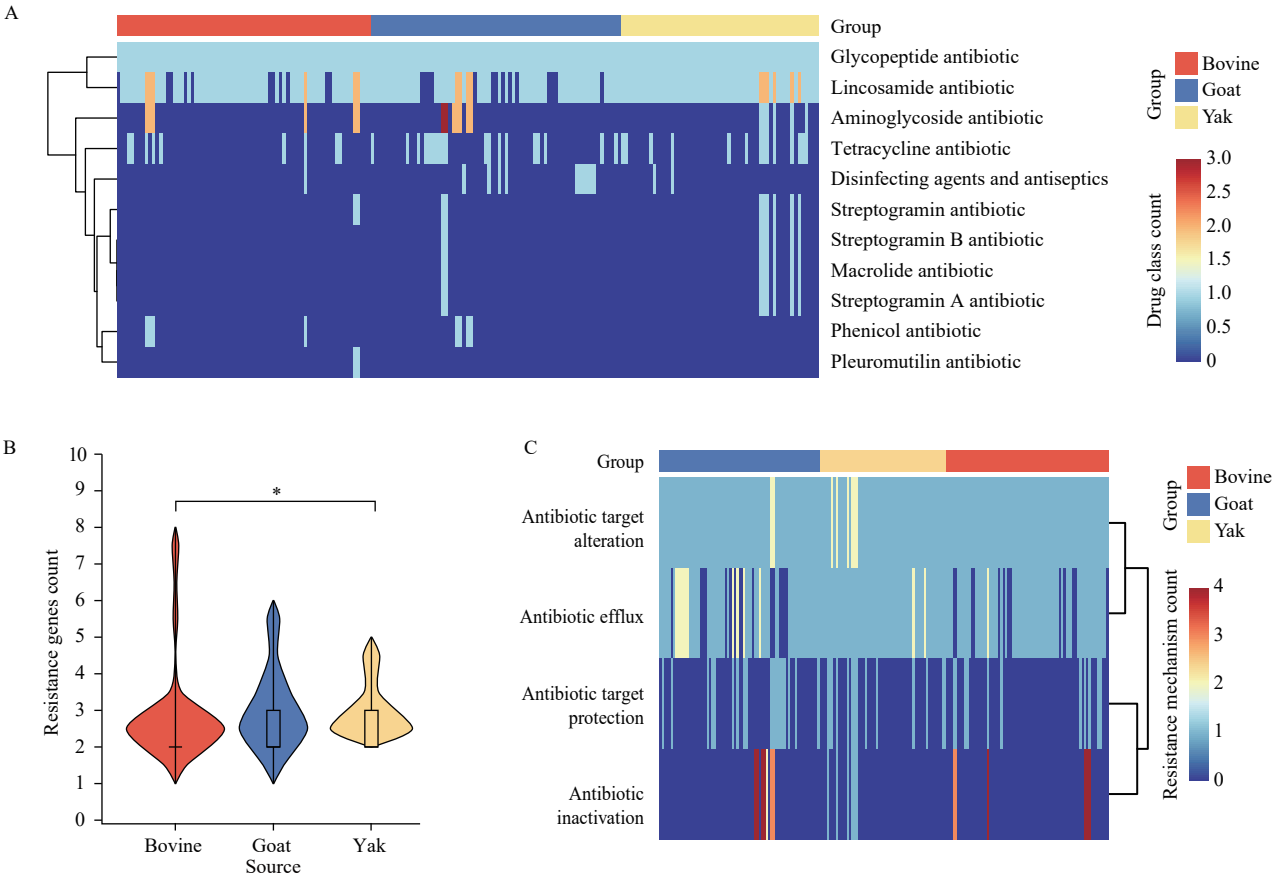

Fig. 6 ARG analysis of *L. lactis* strains. (A) Heatmap showing the distribution of ARG categories annotated in 199 *L. lactis* strains. The heatmap color gradient reflects the number of drug classes, ranging from 0 (blue) to 3 (red), with yellow representing intermediate values. (B) Differences in ARG counts among *L. lactis* isolates from different sources. (C) Heatmap showing the distribution of ARG resistance mechanism annotated in 199 *L. lactis* strains. The heatmap color gradient reflects the number of resistance mechanism, ranging from 0 (blue) to 4 (red), with yellow representing intermediate values. Data are presented as mean ± SD, with * indicating statistical significance at $P < 0.05$.

was enriched in a subset of strains. These results highlight potential differences in safety and adaptive traits among strains from different milk sources.

4. Discussion

L. lactis is widely used in the food industry for its key roles in fermentation, flavor development, and food preservation. Recent studies have primarily focused on its practical applications, such as yogurt fermentation and cheese production[31-33]. Although emerging evidence indicates that strains from different milk sources contribute variably to flavor metabolites in fermented dairy products[34], the genetic mechanisms underlying these strain-specific differences remain largely unresolved. Advances in genomics now provide powerful tools to investigate intraspecific diversity and link genetic variation to functional traits[35-36]. In this study, we systematically analyzed the genomes of 199 strains from different milk sources and applied comparative and functional genomics to elucidate the genetic diversity and environmental adaptation of the species.

The genome size of *L. lactis* is shaped by ecological adaptation, with industrial strains often carrying larger plasmid-rich genomes that enhance lactose utilization in milk[37]. Comparative analysis of strains from different milk sources revealed that goat milk isolates exhibited significantly smaller genome sizes and pangenome counts than strains from other sources, likely reflecting the lower lactose content of goat milk and a reduced need to maintain extensive carbohydrate metabolic pathways[13,38]. The fewer unique genes in yak milk strains likely reflect the relatively closed fermentation environment and lower microbial diversity, whereas the more heterogeneous fermentation conditions of bovine milk strains contribute to greater genetic variability and a higher number of strain-specific genes. Although strains from different milk sources display considerable genomic diversity, phylogenetic analysis indicates that *L. lactis* does not form clades strictly according to strain origin, highlighting the high conservation of core genes within the species. Consistent with our findings, *Lactobacillus helveticus* strains from different geographic origins are also dispersed across multiple evolutionary branches[39].

The process of environmental adaptation may involve gene acquisition and loss[40]. Although the functions of many genes remain unknown, a large number of proteins can be assigned to functional categories of orthologous groups based on sequence similarity to functionally characterized proteins[41-42]. Consistent with previous functional analyses of *Lactococcus* species, the majority of genes in *L. lactis* are annotated to metabolism-related COG categories, including the transport and metabolism of carbohydrates, amino acids, and nucleotides[43-44]. Notably, compared with strains from other sources, bovine milk strains exhibit a higher abundance of genes associated with lipid transport and metabolism, among which COG1835 is particularly prominent. COG1835 is predicted to encode an acyltransferase, which catalyzes the transfer of acyl groups from fatty acids to alcohol molecules, thereby generating ester compounds that contribute to food flavor[45]. For example, the acyltransferase from *L. plantarum* can catalyze the conversion of ethanol to ethyl acetate, imparting a fruity aroma to the product[46]. The enrichment of such genes in bovine milk strains may facilitate more efficient utilization of lipid compounds in the dairy environment and promote the formation of flavor-active metabolites. Carbohydrate metabolism provides lactic acid bacteria not only with energy during dairy fermentation but also with precursor substances and metabolites that directly impact cell growth, fermentation performance, and flavor compound production[47-48]. The pentose and glucuronate interconversions pathway, which mediates the conversion between pentoses and glucuronic acid, has been reported to facilitate the utilization of complex carbohydrates and enhance bacterial adaptability[49]. This pathway was most enriched in *L. lactis* strains isolated from yak milk, reflecting their adaptation to environments containing complex carbon sources.

The distribution of carbohydrate metabolism genes exhibits significant variation among lactic acid bacteria, and the presence or absence of these genes may reflect their adaptation to the availability of growth substrates in different ecological niches[47]. Previous comparative studies on other lactic acid bacteria have demonstrated a strong association between strain origin and carbohydrate metabolism[50-51]. Kefir-derived *L. kefiranofaciens* strains encode more GH31, GH36, and GH42 enzymes for cellulose metabolism than isolates from koumiss or yogurt, indicating stronger plant substrate fermentation capacity and adaptation to a complex niche[26]. Our study revealed that the carbohydrate utilization capacities of *L. lactis* strains from different milk sources varied significantly, particularly in the CE family. The CE family is mainly responsible for removing esterified substitutions on polysaccharides, thereby cooperating with other GHs to degrade complex polysaccharides[52]. β-Galactosidase, which plays a key role in dairy production by hydrolyzing lactose, is mainly classified into the GH1, GH2, and GH42 families, with only specific members of GH1 and GH2 naturally utilizing lactose as a substrate[53]. The enrichment of GH1 and GH2 families among strains from different sources suggests distinct strategies for carbohydrate utilization. GH2 enzymes, with broader substrate specificity than GH1, target lactose as well as terminal β-galactosidic linkages in complex oligosaccharides[54]. The higher abundance of GH2 genes in yak milk-derived *L. lactis* strains likely reflects adaptation to complex carbon sources. We analyzed the *lacZ* genes encoding β-galactosidases within the GH2 family. The results showed a trend toward a higher copy number of the *lacZ* gene in yak milk strains, but this difference did not reach statistical significance, which may be attributable to the limited sample size. A larger sample size or further experimental validation will be required to substantiate this observation. However, comparative SNPs analysis between yak milk and bovine milk strains revealed an amino acid substitution in β-galactosidase, where alanine was replaced by valine at a specific site in the yak milk isolates. Although both alanine and valine are nonpolar amino acids, valine possesses a bulkier side chain. This substitution may alter enzyme activity, substrate affinity, or thermal stability by affecting local protein conformation or the stability of the hydrophobic core, although this hypothesis requires experimental validation.

Phylogenetic and functional analyses suggest that *L. lactis* adapts to different dairy environments mainly through the gain and loss of functional genes, rather than via vertical evolution of the core genome. This may account for the considerable variation in metabolic capacities observed among strains from different sources, despite their highly conserved core genomes. Horizontal gene transfer (HGT), largely mediated by mobile genetic elements (MGEs) such as genomic islands, plasmids, and bacteriophages, is a key mechanism driving this adaptability[55-56]. For example,

integrated conjugative elements in *L. lactis* plasmids confer crucial industrial functions, encompassing phage resistance, antimicrobial compound production, and carbohydrate utilization[57]. A phylogenomic and ancestral analysis based on *L. lactis* strains has shown that ancestral plant-associated lineages diversified into dairy-adapted lineages through extensive HGT, primarily facilitated by MGEs. Pan-genome analyses further reveal substantial diversity in accessory genes and intergenic regions, with habitat-specific enrichment: dairy lineages are enriched in MGEs and CAZymes, whereas plant isolates exhibit reduced genetic exchange[29]. Consistent with these observations, our results demonstrate that strains isolated from different dairy sources also exhibit significant functional differences, particularly in genes related to carbohydrate metabolism and lipid utilization, highlighting the role of the accessory genome in shaping strain-specific adaptations within dairy environments.

Although *L. lactis* has been recognized as GRAS, its ARGs may still be transferred to human gut microbiota via consumption of related fermented products, posing potential health risks[58]. Therefore, a comprehensive understanding of the antibiotic resistance profile of *L. lactis* is crucial for evaluating its safety in food production applications. Glycopeptide resistance genes were the most prevalent in *L. lactis*, indicating potential vancomycin resistance, although this requires further validation. Genes conferring resistance to aminoglycoside and macrolide antibiotics were present in only a few *L. lactis* strains and showed no significant association with strain origin, potentially reflecting strain-specific characteristics. Consistent with our findings, Guo et al.[59] evaluated 33 *Lactobacillus* strains isolated from dairy products and reported that all strains were resistant to vancomycin but susceptible to gentamicin and erythromycin. Another study analyzing the antibiotic resistance of *L. lactis* strains found that all strains were sensitive to gentamicin and kanamycin[60]. In addition, yak milk strains harbored a greater number of annotated ARGs, which may be attributed to the complex production environment of yak milk cheeses, where plasmids and HGT are frequent. Overall, these findings offer crucial evidence for the safe application of *L. lactis*.

5. Conclusion

This study provides a systematic analysis of the genomic diversity and nutritional adaptation of 199 *L. lactis* strains. Significant source-dependent differences were identified in genomic features and functional genes, with yak milk isolates displaying the highest genetic diversity and enrichment of special carbohydrate metabolism genes, thereby supporting broader substrate utilization. Antibiotic resistance profiling additionally revealed the universal presence of glycopeptide resistance genes and multiple resistance mechanisms. These findings highlight the genetic diversity and adaptive evolution of *L. lactis* and identify specific genes, including CE-family enzymes, as potential molecular markers for selecting industrial strains with improved carbohydrate utilization.

Data availability statement

The whole-genome sequencing datasets analyzed in this study can be accessed from the China National GeneBank DataBase (CNGBdb) with accession numbers CNP0007750.

Conflict of interest

Qixiao Zhai is an associate editor for *Food Science and Human Wellness* and was not involved in the editorial review or the decision to publish this article. The authors declare no competing financial interest.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2025.9250789.

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