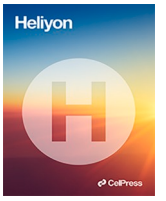
Heliyon 10 (2024) e29499

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Unlocking the genetic potential of *Lacticaseibacillus rhamnosus* strains: Medical applications of a promising probiotic for human and animal health

Monwadee Wonglapsuwan [a], Nuntiya Pahumunto [b,c], Rawee Teanpaisan [d,**], Komwit Surachat [e,f,*]

[a] Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand
[b] Research Center of Excellence for Oral Health, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand
[c] Department of Oral Diagnostic Sciences, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand
[d] Medical Science Research and Innovation Institute, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand
[e] Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand
[f] Translational Medicine Research Center, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand

ARTICLE INFO

Keywords:
Lacticaseibacillus rhamnosus
Probiotic strain
Oral health
Genomics analysis
Comparative genome analysis

ABSTRACT

Lacticaseibacillus rhamnosus is a group of probiotic strains that have gained popularity for their potential health benefits such as promoting digestive health, boosting the immune system, improving lactose digestion, preventing and treating antibiotic-associated diarrhea, reducing the severity and duration of certain infections, and preventing the formation of dental plaque. In particular, *L. rhamnosus* strains SD4 and SD11 have promising human and animal health applications due to their ability to inhibit the growth of harmful pathogens. This study presents an *in silico* genomic analysis of *L. rhamnosus* strains SD4 and SD11. We analyzed draft genomes and conducted comparative genome analyses against several other probiotic strains, aiming to gain insights into the genomes of the two strains and to compare them to related strains isolated from other sources. We also aimed to clarify the functional mechanisms and adaptation of these strains to specific environments. Comprehensive insights into the genomes of *L. rhamnosus* SD4 and SD11 could enhance our understanding of their capacity to colonize, adapt, and exhibit probiotic properties after administration. This study holds significance in advancing our understanding of the potential health benefits associated with these strains and in elucidating the underlying mechanisms responsible for their effectiveness in humans and animals.

1. Introduction

Lacticaseibacillus rhamnosus, formerly known as *Lactobacillus rhamnosus* [1], has gained popularity as a probiotic because of the numerous health benefits that are attributed to it. This species is found naturally in the human gut and has been extensively studied for

* Corresponding author. Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand.
** Corresponding author.
E-mail addresses: rawee.t@psu.ac.th (R. Teanpaisan), komwit.s@psu.ac.th (K. Surachat).

https://doi.org/10.1016/j.heliyon.2024.e29499
Received 26 August 2023; Received in revised form 5 April 2024; Accepted 9 April 2024

Abbreviations

CRISPR	clustered regularly interspaced short palindromic repeat
BLAST	Basic Local Alignment Search Tool
RefSeq	Reference Sequence (database)
CDS	coding DNA sequence
AMR	antimicrobial resistance
VFDB	Virulence Factor Database
CARD	Comprehensive Antibiotic Resistance Database
GI	genomic island
MGE	mobile genetic element
IS	insertion sequence
ANI	average nucleotide identity

its ability to promote digestive health [2] and boost the immune system [3]. *L. rhamnosus* is known for its unique ability to survive in the harsh acidic environment of the stomach, which allows it to reach the intestine and colonize the gut. This probiotic strain has been studied for its potential to improve lactose digestion [4]. It has also shown effectiveness in decreasing both the duration and frequency of diarrhea episodes [5]. Additionally, it may reduce the severity and duration of certain infections [2]. Furthermore, it has demonstrated effectiveness in preventing the formation of dental plaque, a sticky biofilm that leads to tooth decay and gum disease [6]. Overall, *L. rhamnosus* is a promising probiotic with numerous potential health benefits that can help support overall health.

L. rhamnosus has shown promise for various applications in human and animal health [7–9]. In humans, it has been extensively studied for its potential benefits to gastrointestinal health, immune support, allergy prevention, women's health (specifically vaginal health), and oral health. It promotes health and well-being in humans and animals. In particular, *L. rhamnosus* GG is a probiotic strain that has been extensively investigated for its potential health benefits, such as ameliorating diarrhea [7], allergies [8], and respiratory infections [9]. It has also shown a positive effect on gut health by modulating gut microbiota and enhancing intestinal barrier function. Apart from *L. rhamnosus* GG, the probiotic properties of *L. rhamnosus* strains SD4 and SD11 have previously been characterized. For example, *L. rhamnosus* SD4 and SD11 have been studied for their use as potential probiotics in swine feed [10]. These strains exhibited a high inhibitory effect against pathogens such as *Escherichia coli*, *Salmonella enterica*, and *Streptococcus suis* and elicited an increased expression of host defense peptides without any hemolytic activity. In addition, these two strains have been investigated for their ability to boost the production of short chain fatty acids in their hosts; these acids have potential anti-cancer effects, particularly in colorectal cancer prevention [11]. Furthermore, several properties of SD4 and SD11 have been tested including their abilities to induce the expression of human β-defensins-2–4, interleukin-1β, interleukin-6, interleukin-8, and tumor necrosis factor-α in human gingival epithelial cells [12] and to reduce inflammatory cytokine levels and apoptosis markers. Despite the research on the phenotypic characteristics of *L. rhamnosus* SD4 and SD11, there is still a need to examine their genomic profiles and compare them with that of the commonly used probiotic *L. rhamnosus* GG to gain a comprehensive understanding of the diverse phenotypes and functions of these strains.

This study presents an *in silico* genomic analysis of *L. rhamnosus* strains SD4 and SD11. We analyzed draft genome sequences and conducted comparative genome analyses against several other probiotic strains, aiming to gain insights into the genomes of the two strains and to compare them to related strains isolated from other sources. We also aimed to clarify the functional mechanisms and adaptation of these strains to specific environments. Comprehensive insights into the genomes of *L. rhamnosus* SD4 and SD11 could improve our understanding of their capacity to colonize, adapt, and exhibit probiotic properties after administration.

2. Materials and methods

2.1. Ethic statement

This research has been granted exemption from the Human Research Ethics Committee (HREC) of Prince of Songkla University, Thailand (REC.66-200-38-2, dated May 9, 2023).

2.2. Bacterial strains, genomic DNA extraction, and whole-genome sequencing

Two potential probiotic strains, *L. rhamnosus* SD4 and SD11, were isolated from previous study [13] at the Faculty of Dentistry, Prince of Songkla University, Thailand. A single colony of each strain was cultivated in MRS medium (Difco BD, New Jersey, USA) at 37 °C for 24 h under anaerobic conditions. Genomic DNA was extracted using a TIANamp Bacterial DNA Kit (Tiangen, Beijing, China) following the manufacturer's instructions. DNA concentration and quality were determined using a NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). DNA integrity and purity were evaluated using agarose gel electrophoresis. Finally, the extracted DNA was sent for library preparation, and 150 bp paired-end reads were sequenced with BGISEQ-500 (BGI, Shenzhen, China).

2.3. Genome assembly, annotation, and visualization

One gigabase pair of 150 bp paired-end reads per sample was generated from the sequencer in FASTQ format. We then used the automated bioinformatics pipeline, Bacseq v1.0.0 [14], to analyze raw sequence data. This tool is capable of performing *de novo* assembly and annotation of genes, tRNA, and rRNA in bacterial genomes using SPAdes v3.15.5 [15], Prokka v1.12 [16], tRNAscan-SE [17] and RNAmmer [18], respectively. Furthermore, we screened the sequenced DNA for the presence of plasmids using an *in silico* method as described previously [19]. CRISPRFinder [20] was used to identify clustered regularly interspaced short palindromic repeats (CRISPRs). Circular genome representations were visualized Proksee [21].

2.4. In silico safety assessment

In the field of bacterial safety assessment, *in silico* methods are important tools for predicting the risks associated with bacterial strains. This approach employs computer-based models and tools to analyze genomic content and identify potential pathogenicity, virulence factors, and antimicrobial resistance (AMR). A range of specialized tools are available to conduct such assessments. PathogenFinder [22] was used to predict the pathogenic potential, while ResFinder [23] and Comprehensive Antibiotic Resistance Database (CARD) [24] were used to predict AMR genes in bacterial genomes. In contrast, VFanalyzer and Virulence Factor Database (VFDB) [25], were used to identify virulence factors and potential virulence genes in bacterial genomes. By utilizing these tools in combination, bacterial strains can be evaluated for safety before being used in food production or as probiotics. The results of *in silico* safety assessment can guide further experimental work to confirm the safety of bacterial strains and identify potential risks.

2.5. Identifying genes related to probiotic features

Genes identified from literature searches to belong to the genus *Lactobacillus* [26–29] are involved in important biological processes, such as adhesion mechanisms, resistance to various stress conditions, repair and protection of DNA and proteins, and vitamin production. To identify similar genes in the genome of interest, we performed protein sequence alignments using the Basic Local Alignment Search Tool (BLAST). A cutoff of 1E-20 and a minimum identity percentage of 70 % were used to ensure the accuracy and reliability of the results. This approach provides insights into the potential functional roles of these genes in probiotic features. Additionally, the process of identifying genes responsible for the production of ribosomally synthesized and post-translationally modified peptides and bacteriocins was carried out using BLASTP to perform a sequence similarity search against the BAGEL database [30]. Gene clusters that were deemed relevant underwent further analysis and visualization using the BAGEL4 web server [31]. AntiSMASH [32] was employed to detect and examine the gene clusters responsible for secondary metabolite biosynthesis in the bacterial genomes.

2.6. Genomic plasticity analysis

Phigaro v2.4.0 [33] was used to detect and annotate the prophage regions present in the genome. Additionally, MobileElementFinder v1.0.3 [34] was used to identify mobile genetic elements (MGEs). IslandViewer4 [35] was used to predict clusters of genes in the bacterial genome that were possibly obtained via horizontal gene transfer.

2.7. Comparative genomic analysis

We compared *L. rhamnosus* strains SD4 and SD11 with three other probiotic strains: *L. rhamnosus* GG [36], *L. rhamnosus* Pen [27], and *L. rhamnosus* Gr-1n [37]. We used the BLAST function on the Proksee server to visualize the identity of the coding sequences across all strains. In addition, we selected *L. rhamnosus* SD4 as the central ring for our analysis.

2.8. Pan-genome analysis

In this study, 243 *L. rhamnosus* genomes were obtained from the NCBI Reference Sequence Database (RefSeq). To eliminate potential bias resulting from different annotation protocols, all 243 genomes were re-annotated using Prokka 1.14.6 [16]. The resulting general feature format files were then utilized in pan-genome analysis, which involved identifying core, accessory, and unique protein families using Roary [38], with a 95 % BLASTP threshold and standard parameters. The bacterial strains used in the pan-genome analysis are shown in Table S1. A total of 1160 core genes from 245 bacterial strains (including SD4 and SD11) were used for multiple alignments using MUSCLE [39], followed by the construction of phylogenetic trees using MEGA software [40] and the neighbor-joining method. Bootstrap testing with 1000 repetitions was used to assess tree reliability.

3. Results and discussion

3.1. Characteristics of the L. rhamnosus SD4 and SD11 genomes

The SD4 and SD11 genomes had a combined size of 3.07 and 2.82 Mbp and a GC content of 46.6 % and 46.7 %, respectively, as shown in Fig. 1A and B. SD4 and SD11 contained 2898 and 2609 protein coding DNA sequences (CDSs), with average lengths of 883

and 901 bp, respectively. These protein CDSs constituted 83.2 % of the genome. Further information regarding the genomic statistics of the two strains is presented in Table 1.

Among the CDSs identified in strains SD4 and SD11, 1163 (36.9 %) and 959 (34.1 %) genes, respectively, were hypothetical. The classifications of ORFs based on the SEED subsystem categorization from the RAST server and Clusters of Orthologous Groups database are presented in Fig. 2A and B, respectively. The SEED subsystem classifications of SD4 and SD11 were grouped into 25 subsystems, excluding only "Motility and Chemotaxis" and "Photosynthesis." The most abundant subsystems in both strains were carbohydrates, protein metabolism, and amino acids and their derivatives, indicating the presence of genes associated with these metabolic pathways. The identification of carbohydrate metabolism genes in SD4 and SD11 is important, because they are involved in fermenting sugars and producing lactic acid during the fermentation of dairy products and other foods. This study contributes to a better understanding of the probiotic properties of these strains. In addition to the SEED subsystem classifications, a higher number of subsystems related to phages, prophages, transposable elements, and plasmids were identified in SD4 (67) than in SD11 (7), which could be attributed to the larger size of the SD4 genome. This finding suggests that the larger genome of SD4 may provide more opportunities for the acquisition and integration of MGEs, such as phages and plasmids, compared to SD11. MGEs can play a significant role in bacterial evolution, as they can transfer genes between different strains and species, including those involved in virulence and AMR. The larger number of subsystems identified in SD4 is consistent with previous studies on bacterial genomes [41,42], which have shown that larger genomes tend to have a higher abundance and diversity of MGEs. However, it is important to note that the size difference between SD4 and SD11 may not be the only factor contributing to the observed differences in the number of identified subsystems.

3.2. *In silico safety assessment*

Both strains, SD4 and SD11, exhibited no AMR genes in ResFinder 4.1 and CARD. Furthermore, the probability of these strains being human pathogens was calculated using the PathogenFinder tool, and the results were 0.186 for SD4 and 0.184 for SD11. These values suggested that neither SD4, nor SD11 are human pathogens [43]. No virulence genes were detected in either genome using a BLASTn search of the VFDB. This suggests that SD4 and SD11 are safe and do not pose a risk as human pathogens. The absence of AMR genes in ResFinder and CARD is also a positive indication, because AMR is a significant public health concern. The absence of virulence genes in the VFDB further supports the notion that these strains are non-pathogenic. CRISPR regions have been identified in the SD4 and SD11 genomes and play a crucial role in providing immunity to bacteria against invading genetic elements [44,45]. CRISPR systems are an essential defense mechanism in bacteria against MGEs, such as bacteriophages, transposons, and plasmids, which can carry AMR and virulence genes. The presence of CRISPR regions in the SD4 and SD11 genomes indicates that these bacteria could defend against such a potential threat. Overall, the absence of AMR genes, virulence genes, and the low probability of being a human pathogen indicate that SD4 and SD11 are safe for use as probiotics. These findings are relevant in the context of increasing concerns regarding AMR and the need to identify safe and effective probiotics for human use.

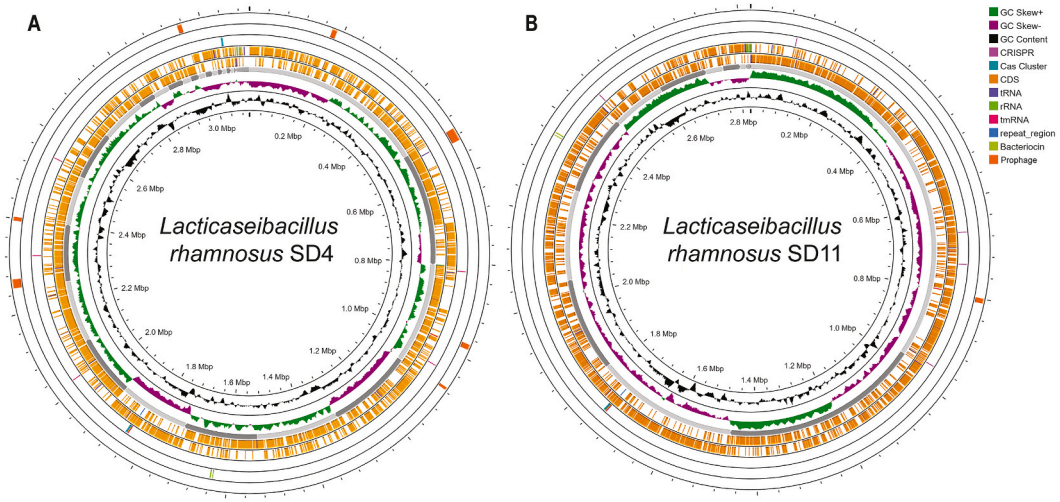


Fig. 1. The circular genome map of *L. rhamnosus* strains SD4 (A) and SD11 (B) displays the genomic features in an organized manner. The outermost ring represents the prophage region (in orange), followed by the bacteriocin-encoding gene (in lime), the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas cluster (in violet and teal), coding DNA sequences (CDSs) on the forward and reverse strands (in orange peel), and the positive (in green) and negative (in dark violet) GC skew, with the GC content shown in black. Additionally, non-coding RNA and transfer-messenger RNA (tmRNA) are also included in the CDS ring. tRNA, transfer RNA; rRNA, ribosomal RNA. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Genome statistics of *L. rhamnosus* strains SD4 and SD11.

Genomic features	SD4	SD11
Genome size (bp)	3,074,301	2,824,505
GC content (%)	46.5	46.7
Number of contigs	71	26
Longest contig size	503,140	973,035
N50	197,258	489,555
Number of CDSs	2898	2609
tRNA	57	58
rRNA	8	5
tmRNA	1	–
ncRNA	2	2
Repeat region	1	1
Regulatory gene	11	11
Number of subsystems	231	226

Abbreviations: CDS, coding DNA sequence; tRNA, transfer RNA; rRNA, ribosomal RNA; tmRNA, transfer-messenger RNA; ncRNA, non-coding RNA.

3.3. Probiotic properties

L. rhamnosus strains contain a large number of genes that encode proteins involved in stress responses, which help them adapt to the gastrointestinal tract and oral cavity. These stress responses are related to factors such as temperature, pH, bile, osmotic pressure, and oxidative stress. To determine the probiotic functions of the SD4 and SD11 strains at the genomic level, we searched for various probiotic property-related genes, including those involved in stress resistance, bile salt hydrolase activity, adhesion ability, and immunomodulatory activities, based on previously published literature. Our analysis revealed several genes encoding stress-related proteins in the genomes of both strains, which are listed in Table 2.

L. rhamnosus is well known for its ability to adhere to and colonize both the gastrointestinal and oral mucosa [46], which is attributed to a variety of adhesion mechanisms that involve numerous genes. Adhesion-related genes identified in strains SD4 and SD11 include *strA*, *malP*, *fbp*, *lspA*, *tuf*, and *gpr* [36,47]. These genes contribute to the complex adhesion mechanisms of *L. rhamnosus* by mediating the interactions between host cells and extracellular matrix components, promoting Exopolysaccharide production, and regulating adhesion-related gene expression and cell surface properties. These mechanisms enable *L. rhamnosus* to colonize the gastrointestinal tract and potentially provide health benefits. In a previous study [10], we found that *L. rhamnosus* SD4 and SD11 exhibit strong adhesion to Caco-2 cells, a human intestinal epithelial cell line, which may be mediated by various adhesion-related genes (Table 2).

L. rhamnosus is resistant to various stressors, including high temperature, low pH, bile salts, and oxidative stress, which is essential for its survival and adaptation to the host gastrointestinal tract. Heat stress can result in damage to proteins and cellular components; however, *L. rhamnosus* has various mechanisms that counteract this stress. One crucial mechanism involves the regulation and activation of the chaperone and protease genes responsible for the refolding and degradation of misfolded or damaged proteins. In our study, we discovered the *clp* operon (Table 2), which includes the Clp family of proteins, including ClpB, ClpC, ClpE, ClpL, ClpX, and ClpP. These proteins are involved in folding, refolding, and degradation [48,49]. They play an essential role in preserving cellular homeostasis during heat stress by regulating protein quality and removing damaged proteins. Other heat shock proteins involved in SD4 and SD11 responses to heat stress include HslV, HrcA, HslO, HSP20, DnaK, DnaJ, CtsR, GrpE, GroEL, GroES, RuvA, RecA, and LexA. In addition, these proteins are involved in the repair and protection of damaged proteins, DNA damage repair, and regulation of gene expression.

L. rhamnosus can synthesize certain B-group vitamins, including vitamins B12 (cobalamin) and B9 (folate) [50]. Vitamin B12 biosynthesis in SD4 and SD11 strains involves a set of genes, including the *cob* operon, which is responsible for the conversion of cobinamide to cobalamin, and the corrinoid salvage pathway, which allows bacteria to use exogenous cobalamin as a vitamin source. Folate production in *L. rhamnosus* is also mediated by a set of genes, including *folA*, which is responsible for dihydrofolate synthesis, and *folE*, which is involved in the production of tetrahydrofolate, the active form of vitamin B9. The ability of *L. rhamnosus* to synthesize these vitamins can have potential health benefits for the host, as they are important for various metabolic processes [51,52].

Additionally, *L. rhamnosus* produces various bacteriocins and secondary metabolites with potential probiotic and health-promoting properties. Bacteriocins are antimicrobial peptides that inhibit the growth of other bacteria, including pathogenic ones. *L. rhamnosus* can produce several bacteriocins, including rhamnosin A [53], which exhibit antimicrobial activity against pathogenic bacteria, such as *Staphylococcus aureus* and *Listeria monocytogenes*. In this study, we identified three bacteriocin-encoding genes in SD4 (Fig. 3A) and SD11 (Fig. 3B) strains using the BAGEL5 server, as shown in Table 2. Although there is limited evidence to suggest that *L. rhamnosus* can generate enterocin X, a previous study aimed to identify and describe a possible bacteriocin produced by *L. rhamnosus* L156.4 [54]. Class II bacteriocins were identified in the genome using the BAGEL3 server. We reanalyzed the results using the same software version and obtained the same list of bacteriocins as those discovered in the SD4 and SD11 genomes. Oliveira et al. (2017) showed that L156.4 has a broad antibacterial spectrum against various pathogenic and spoilage bacteria, including *E. coli*, *Bacillus cereus*, *S. aureus*, *L. monocytogenes*, and *S. enterica*. These results corroborate those of our previous work [10], which showed that SD4 and SD11 can inhibit several pathogens, including *E. coli*, *S. enterica*, and *S. suis*. The three bacteriocin-encoding genes identified in this study may be

Fig. 2. Functional classifications of open reading frames based on SEED subsystem categorization (A) and Clusters of Orthologous Groups (COG) identification (B) for the SD4 and SD11 genomes.

Table 2
Probiotic genes identified in the SD4 and SD11 genomes.

Adhesion			
Gene	Function	SD4	SD11
fbp	Fructose-1,6-bisphosphatase class 3	+	+
lspA	Lipoprotein signal peptidase	+	+
tuf	Elongation factor Tu	+	+
gpr	L-glyceraldehyde 3-phosphate reductase	+	+
luxS	S-ribosylhomocysteine lyase	+	+
ywpE	Putative sortase YwpE	+	+
tpiA	Triosephosphate isomerase	+	+
gapA	Glyceraldehyde-3-phosphate dehydrogenase	+	+
eno	Enolase	+	+
pgi	Glucose-6-phosphate isomerase	+	+
epsH	Putative glycosyltransferase EpsH	+	+
malP	Maltose phosphorylase	+	+
strA	Sortase A	+	+
cpsY	Exopolysaccharide phosphotransferase CpsY	+	–
arnC	Undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase	+	–
pglC	Undecaprenyl phosphate N,N′-diacetylbacillosamine 1-phosphate transferase	+	–
wbbI	Beta-1,6-galactofuranosyltransferase WbbI	–	+
glyD	Glycosyltransferase GlyD	–	+
cpsY	Exopolysaccharide phosphotransferase CpsY	–	+
epsF	Putative glycosyltransferase EpsF	–	+
Stress resistance			
Gene	Function	SD4	SD11
Heat stress			
clpB	ATP-dependent Clp protease ATP-binding subunit ClpB	+	+
clpC	ATP-dependent Clp protease ATP-binding subunit ClpC	+	+
clpE	ATP-dependent Clp protease ATP-binding subunit ClpE	+	+
clpL	ATP-dependent Clp protease ATP-binding subunit ClpL	–	–
clpX	ATP-dependent Clp protease ATP-binding subunit ClpX	+	+
clpP	ATP-dependent Clp protease ATP-binding subunit ClpP	+	+
hslV	ATP-dependent protease subunit HslV	+	+
hrcA	Heat-inducible transcription repressor HrcA	+	+
hslV	ATP-dependent protease subunit HslV	+	+
hslO	Molecular chaperone Hsp33	+	+
HSP20	HSP20 family protein	+	+
dnaK	HSPA9; molecular chaperone DnaK 1	+	+
dnaJ	Molecular chaperone DnaJ	+	+
ctsR	Transcriptional regulator CtsR	+	+
grpE	Protein GrpE	+	+
groEL	Chaperonin GroEL	+	+
groES	Chaperonin GroES	+	+
ruvA	Holliday junction ATP-dependent DNA helicase RuvA	+	+
recA	Protein RecA	+	+
lexA	LexA repressor	+	+
Cold stress			
Csp	Cold shock protein 1	+	+
cspLA	Cold shock-like protein CspLA	+	+
cspB	Cold shock protein CspB	+	+
Nox	NADH oxidase	+	+
Npr	NADH peroxidase	+	+
trxA	Thioredoxin	+	+
trxB	Thioredoxin reductase	+	+
Acid stress			
atpA	ATP synthase subunit alpha	+	+
atpB	ATP synthase subunit A	+	+
atpC	ATP synthase epsilon chain	+	+
atpD	ATP synthase subunit beta	+	+
atpE	ATP synthase subunit C	+	+
atpF	ATP synthase subunit B	+	+
atpG	ATP synthase gamma chain	+	+
dltB	D-alanyl-lipoteichoic acid biosynthesis protein DltB	+	+
Bile salt tolerance			
cfa	Cyclopropane-fatty-acyl-phospholipid synthase	+	+
ppaC	Putative manganese-dependent inorganic pyrophosphatase	+	+
brpA	Biofilm regulatory protein A	+	+
ltaS1	Lipoteichoic acid synthase 1	+	+

DNA and protein protection and repair			
Gene	Function	SD4	SD11
msrA	Peptide methionine sulfoxide reductase MsrA	+	+
msrC	Free methionine-R-sulfoxide reductase	+	+
msrB	Peptide methionine sulfoxide reductase MsrB	+	+
uvrA	UvrABC system protein A	+	+
clpC	Negative regulator of genetic competence ClpC/MecB	+	+
msrB	Peptide methionine sulfoxide reductase MsrB	+	+
luxS	S-ribosylhomocysteine lyase	+	+
mutT	MutT/nudix family protein	+	+
uvrA	Excinuclease ABC subunit UvrA	+	+
Vitamin biosynthesis			
Gene	Function	SD4	SD11
ribU	Riboflavin transporter RibU	+	+
ribZ	Riboflavin transporter RibZ	+	+
ribF	Bifunctional riboflavin kinase/FMN adenylyltransferase	+	+
cobC	Adenosylcobalamin/alpha-ribazole phosphatase	+	+
cobB	NAD-dependent protein deacetylase	+	+
folA	Dihydrofolate reductase	+	+
folT	Folate transporter FolT	+	+
btuD	Vitamin B12 import ATP-binding protein BtuD	+	+
ytrB	Vitamin B12 import ATP-binding protein BtuD	+	+
lnrL	Vitamin B12 import ATP-binding protein BtuD	+	+
Immunomodulation			
Gene	Function	SD4	SD11
dltA	D-alanine—poly(phosphoribitol) ligase subunit	+	+
dltB	Membrane protein involved in D-alanine export	+	+
dltC	D-alanine—poly(phosphoribitol) ligase subunit	+	+
dltD	D-alanine transfer protein	+	+
-	Isopeptide-forming pilin-related protein SpaA	+	+
-	Cell surface protein SpaB	+	+
-	Aliphatic sulfonate ABC transporter substrate-binding protein SpaC	+	+
Bacteriocin			
Class	Description	SD4	SD11
Bacteriocin_IIa	Carnocin CP52	+	+
Bacteriocin_IIc	Enterocin X chain beta	+	+
Bacteriocin_IIc	Bacteriocin class II with double-glycine leader peptide	+	+
Secondary metabolite biosynthesis			
Class	Description	SD4	SD11
T3PKS	Type III polyketide synthase	+	+
RiPP-like	Other unspecified ribosomally synthesized and post-translationally modified peptide product (RiPP)	+	+

crucial *for L. rhamnosus* strains SD4 and SD11 to exert antimicrobial activity against pathogenic bacteria. Furthermore, we identified *T3PKS* genes and post-translationally modified peptide products in the genomes of *L. rhamnosus* strains SD4 and SD11 using anti-SMASH analysis. *T3PKS* genes have been found in several *Lactobacillus* spp., indicating their ability to synthesize various secondary metabolites. *Lactiplantibacillus plantarum* [55] and *L. rhamnosus* [56] are examples of bacteria carrying *T3PKS* genes. These secondary metabolites have different functions, including antimicrobial activity and signaling. The discovery of these secondary metabolites and their corresponding biosynthetic pathways could provide valuable information on the potential biological activities and applications of the SD4 and SD11 strains.

3.4. Genome plasticity

Genome plasticity refers to the ability of a genome to change in response to environmental pressure over time through various mechanisms, such as mutation, recombination, and horizontal gene transfer [57,58]. This allows bacteria to adapt and evolve in response to changing environmental conditions, such as the presence of antibiotics or other stressors. We used IslandViewer4 to predict the presence of genomic islands (GIs) in the genomes of *L. rhamnosus* SD4 (Fig. 4A) and SD11 (Fig. 4B). Fourteen and six GIs were identified in SD4 and SD11, respectively, ranging in size from 4524 to 56,250 bp and 5703 to 60,563 bp. However, virulence factors or pathogen-associated genes were not detected in either genome. In SD4, most annotated CDSs were hypothetical proteins, followed by phage proteins, transposases, and endonucleases. Similarly, in SD11, hypothetical proteins were the most common CDSs, followed by PTS sugar and fructose transporters.

Overall, the presence of GIs in the genomes of *L. rhamnosus* SD4 and SD11 suggests that horizontal gene transfer events may have

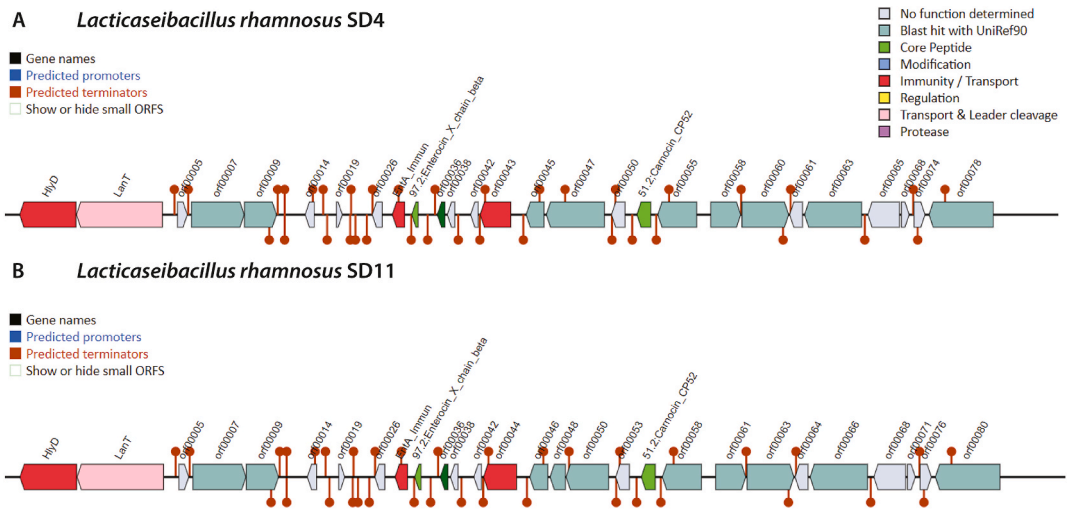


Fig. 3. The arrangement of genetic clusters responsible for the production of bacteriocins in the genomes of *L. rhamnosus* strains SD4 (A) and SD11 (B), as predicted via the BAGEL4 webserver.

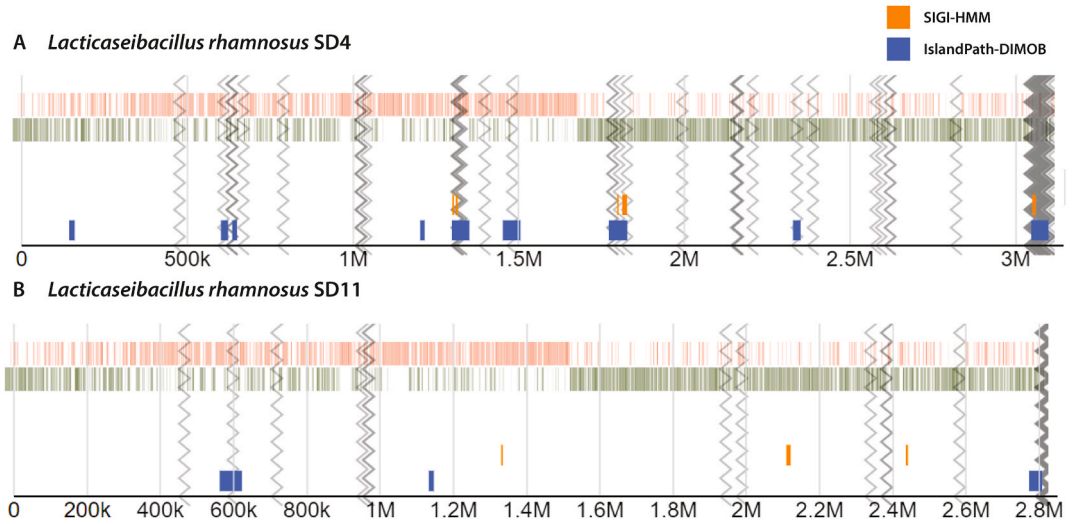


Fig. 4. The arrangement of genomic islands (GIs) in the genomes of *L. rhamnosus* strains SD4 (A) and SD11 (B) determined using the IslandViewer4 prediction tool. Each GI is represented by a different color in the bar, indicating the method used for its prediction. The contig boundary is marked via a zigzag line, and the forward and reverse coding sequences are represented in pink and lime, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

contributed to their genomic plasticity and adaptation to different environments [59,60]. However, the absence of virulence factors and pathogen-associated genes in these islands suggests that they may not play a significant role in the pathogenicity of these strains. The abundance of hypothetical proteins and transporters in the CDSs of both strains implies that they may play important roles in cellular functions such as metabolism and transport. Further studies are required to explore the functions and potential applications of these GIs in *L. rhamnosus*.

Phigaro analysis was conducted to detect the prophage sequences in the genomes of *L. rhamnosus* SD4 (Fig. 5A) and SD11 (Fig. 5B). These findings revealed the presence of seven prophage regions in the SD4 genome and one prophage region in the SD11 genome. Six of the prophage regions in the SD4 genome belonged to the *Siphoviridae* family, whereas the taxonomy of the remaining region remains unknown. Similarly, the prophage region in the SD11 genome belonged to the *Siphoviridae* family. Previous studies have also reported the presence of *Siphoviridae* prophages in *L. rhamnosus* [61,62]

The identification of *Siphoviridae* prophages through Phigaro analysis provided valuable insights into the potential presence of phage-related genetic elements in *L. rhamnosus* strains SD4 and SD11. Prophages play a significant role in the genetic diversity, evolution, and functional characteristics of bacterial populations [63]. Understanding the distribution and classification of prophage sequences will contribute to our knowledge of their involvement in horizontal gene transfer and adaptation to changing environments.

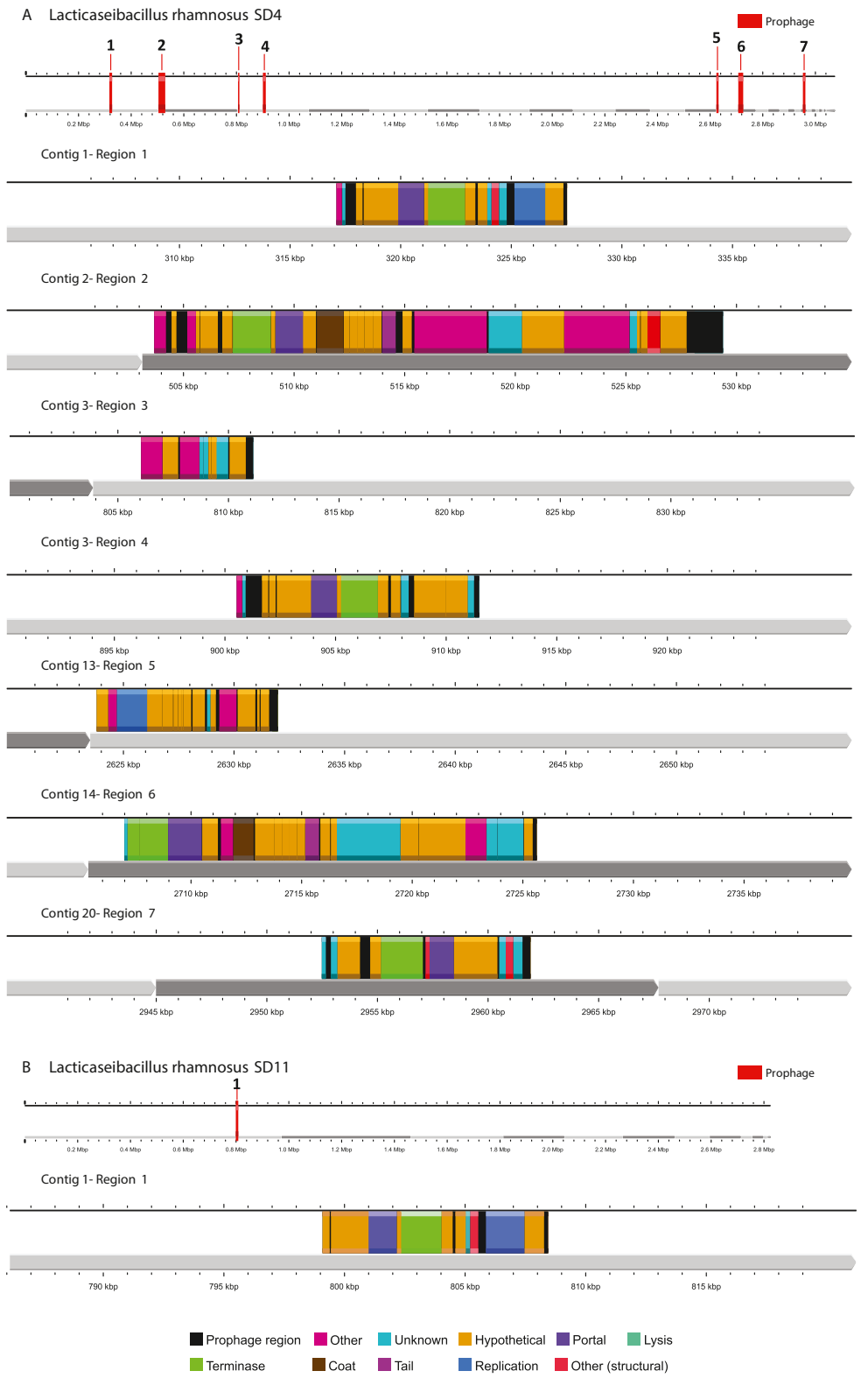


Fig. 5. Prophage regions of *L. rhamnosus* strains SD4 (A) and SD11 (B) identified using Phigaro.

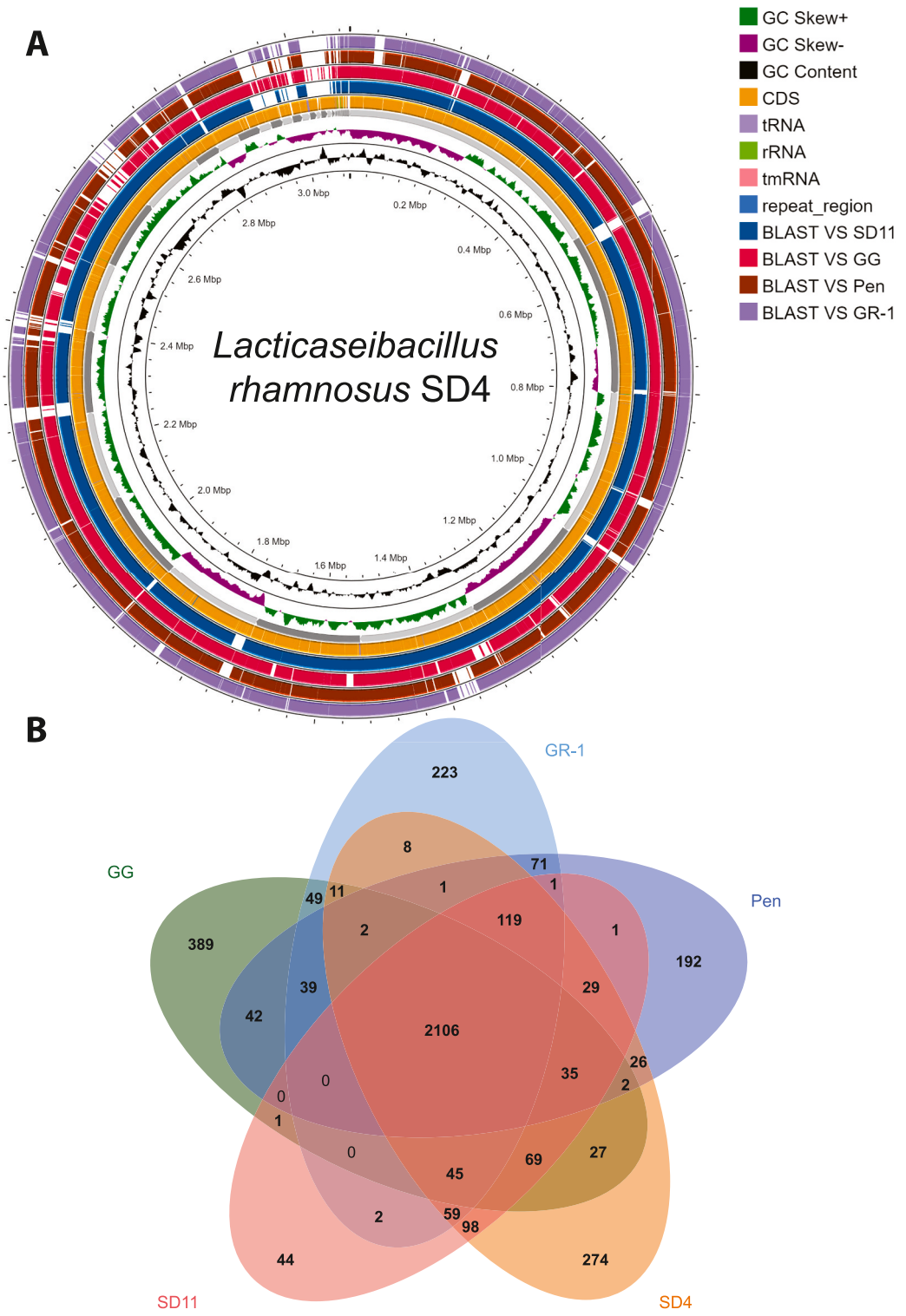


Fig. 6. Comparative genome analysis results. (A) Comparative genome mapping using the Proksee webserver. (B) Venn diagram illustrating the results of the comparative genomic analysis among five *L. rhamnosus* strains. The numbers within the overlapping areas represent the count of orthologs predicted by the Roary pipeline. CDS, coding DNA sequence; tmRNA, transfer-messenger RNA; tRNA, transfer RNA; rRNA, ribosomal RNA, BLAST, Basic Local Alignment Search Tool.

Further investigation and characterization of these prophage regions could provide additional information regarding the genetic landscape and dynamics of *L. rhamnosus* SD4 and SD11.

Furthermore, MobileElementFinder analysis was used to examine the presence of MGEs and insertion sequences (ISs) in the genomes of *L. rhamnosus* SD4 and SD11. The results identified one MGE in the SD4 genome, specifically the IS5 element, which exhibited 100 % alignment coverage and 99.62 % sequence identity. In contrast, MGEs were not detected in the SD11 genome. Overall, the identification of IS5 in the SD4 genome highlights the potential for genetic plasticity and rearrangement in this *L. rhamnosus* strain, whereas the absence of MGEs in the SD11 genome suggests a relatively more stable genetic profile. Further investigation of the functional implications of these MGEs and IS elements in *L. rhamnosus* strains can provide valuable insights into their genetic dynamics, adaptive capabilities, and potential roles in microbial ecology.

3.5. Comparative genomic analysis

We conducted a comparative analysis of the genomes of five *L. rhamnosus* strains using *L. rhamnosus* SD4. The BLAST analysis was visualized using the Proksee server as shown in Fig. 6A. The overall genome composition of most strains was similar. The Venn diagram in Fig. 6B illustrates the presence of both specific and homologous core genes in the five *L. rhamnosus* strains. Specifically, 2106 genes were shared among all strains. *L. rhamnosus* GG had the highest number of unique genes (389), whereas *L. rhamnosus* SD4 had the lowest (44). Furthermore, the average nucleotide identity (ANI) among these five strains was over 97 %. Notably, samples SD4 and SD11 exhibited the highest ANI values (99.75 %). These findings suggest that although the overall genome composition of the five *L. rhamnosus* strains was largely similar, there were specific genes unique to each strain. *L. rhamnosus* GG had a larger number of unique genes than the other strains. High ANI values indicated a close relationship between the analyzed strains, particularly between SD4 and SD11.

Interestingly, all five compared strains had the same genes responsible for encoding bacteriocins, namely carnocin CP52, enterocin X chain beta, and class II bacteriocin with a double-glycine leader peptide. However, *L. rhamnosus* GG had an additional gene encoding the bacteriocin LSEI_2386, which is commonly found in *Lacticaseibacillus casei* [64] and *Lacticaseibacillus paracasei* [65,66]. Although *L. rhamnosus* GG had more bacteriocins than SD4 and SD11, the antimicrobial susceptibility results from our previous study [10] indicated that SD11 and SD4 exhibited higher susceptibility to antimicrobials against enterotoxigenic *E. coli*, non-enterotoxigenic *E. coli*, *S. enterica*, and *S. suis* strains. This suggests that the presence of more bacteriocins in *L. rhamnosus* GG may not necessarily be correlated with higher antimicrobial susceptibility in these strains. These observations highlight the complexity of bacteriocin activity, the interplay between different strains, and their susceptibility to antimicrobials. Further research is required to understand the specific mechanisms and interactions involved in harnessing the potential benefits of these bacteriocins for antimicrobial applications.

Comparative analysis of the genomes provided valuable insights into the genetic diversity and relatedness among the different strains of *L. rhamnosus*. These findings contribute to our understanding of the functional and phenotypic variations between these strains and have implications for further research and applications in the fields of probiotics and microbial ecology.

3.6. Pan-genome analysis

We conducted pan-genome analysis to assess the diversity of *L. rhamnosus* strains by comparing SD4 and SD11 with all available *L. rhamnosus* genomes from the RefSeq database. A total of 245 genomes, including those of the two proposed strains, were analyzed. The results revealed a pan-genome consisting of 12,754 gene clusters, which encompassed a core gene set (9.09 %; 1160 genes), an accessory gene set (56.38 %; 7191 genes), and a strain-specific gene set (34.52 %; 4403 genes).

The core gene set represented the genes shared by all analyzed *L. rhamnosus* strains, exhibiting a gene sequence similarity higher than 95 %. These core genes likely contribute to fundamental biological functions and conserved characteristics of *L. rhamnosus*. The accessory gene set comprised genes that were present in some, but not all of the examined strains. These genes contribute to the genomic diversity and potential functional variations observed between the different *L. rhamnosus* strains. Additionally, the strain-specific gene set comprised genes unique to specific strains, suggesting distinct genetic features and potential strain-specific functionalities.

In addition to assessing the diversity of *L. rhamnosus* strains, we explored the openness of the genome through pan-genome analysis. Interestingly, our findings revealed that the pan-genome of *L. rhamnosus* continued to grow significantly, even after sequencing over 245 genomes. This suggests that *L. rhamnosus* possesses an open genome, indicating that new genes are being discovered with each additional sequenced genome. *L. rhamnosus* is naturally found in various sources, and its ability to adapt to different environments may be attributed to mechanisms such as horizontal gene transfer and genetic mutations. These processes play crucial roles in the acquisition of new genetic material and contribute to the genomic plasticity observed in this species.

These results highlight the need for continued sequencing of the *L. rhamnosus* genome to further explore its genomic diversity and identify new genes. Until the pan-genome of this species reaches a closed state, where most genes are shared among all strains, there is still much to learn about the genetic makeup and potential functionalities of *L. rhamnosus*. This information emphasizes the dynamic nature of the *L. rhamnosus* genome and the ongoing evolution and adaptation processes within this species. Further research and sequencing will contribute to a deeper understanding of its genomic diversity and provide valuable insights for various applications, including the development of probiotics and the study of microbial ecology.

4. Conclusions

Our study sheds light on the potential health benefits of *L. rhamnosus* SD4 and SD11, particularly in human and animal health. By conducting in-depth genomic analyses, we gained valuable insights into the genetic composition and probiotic properties of these strains. Our findings demonstrate that both SD4 and SD11 possess promising characteristics, including the ability to inhibit the growth of harmful bacteria associated with dental plaque and periodontal disease. Moreover, our *in-silico* safety assessment revealed that these strains do not harbor antimicrobial resistance genes or virulence factors, indicating them being safe for human use. Furthermore, our study highlights the probiotic potential of SD4 and SD11, as evidenced by their stress resistance, adhesion ability, and immunomodulatory properties. Through comparative genomic analysis, we observed genetic variations among different *L. rhamnosus* strains, which may influence their probiotic functionalities and applications. Additionally, pan-genome analysis revealed the dynamic nature of the *L. rhamnosus* genome, indicating ongoing evolution and adaptation processes within this species. Overall, our findings contribute to a better understanding of the probiotic properties of *L. rhamnosus* strains SD4 and SD11 and their potential implications for human and animal health. In the future, it is important for research efforts to be concentrated on leveraging the therapeutic capabilities of these strains to create specific interventions aimed at enhancing gastrointestinal, oral well-being and treatment in diabetes.

Funding

This research was funded by the National Science, Research, and Innovation Fund (NSRF) and the Prince of Songkla University, Thailand, grant number MED6601151S.

Data availability statement

Genomic information for *L. rhamnosus* SD4 and SD11 strains is available in BioProject, BioSample, and GenBank. The accession numbers for these strains are PRJNA891106, SAMN31309353-354, JAOXMX000000000, and JAOXMY000000000.

CRediT authorship contribution statement

Monwadee Wonglapsuwan: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Data curation, Conceptualization. **Nuntiya Pahumunto:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis. **Rawee Teanpaisan:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Komwit Surachat:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29499.

References

[1] J.S. Zheng, et al., A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae, Int. J. Syst. Evol. Microbiol. 70 (4) (2020) 2782–2858.
[2] X. Peng, et al., Lacticaseibacillus rhamnosus alleviates intestinal inflammation and promotes microbiota-mediated protection against Salmonella fatal infections, Front. Immunol. 13 (2022) 973224.
[3] S. Dehghani, et al., Effect of Lacticaseibacillus rhamnosus and Lactiplantibacillus plantarum isolated from food and human origin on reduction of IgE-dependent hypersensitivity in Balb/c mice, Immunobiology 227 (6) (2022) 152292.
[4] N. Hussain, et al., Generation of lactose- and protease-positive probiotic Lacticaseibacillus rhamnosus GG by conjugation with lactococcus lactis NCDO 712, Appl. Environ. Microbiol. 87 (6) (2021).
[5] Y.T. Li, et al., Efficacy of Lactobacillus rhamnosus GG in treatment of acute pediatric diarrhea: a systematic review with meta-analysis, World J. Gastroenterol. 25 (33) (2019) 4999–5016.
[6] P. Abikshyeet, et al., Probiotics: dawn of a new era in dental caries management, J. Pharm. BioAllied Sci. 14 (Suppl 1) (2022) S34–S38.
[7] G.L. Banna, et al., Lactobacillus rhamnosus GG: an overview to explore the rationale of its use in cancer, Front. Pharmacol. 8 (2017) 603.
[8] J. Huang, et al., Effect of probiotics on respiratory tract allergic disease and gut microbiota, Front. Nutr. 9 (2022) 821900.
[9] S. Liu, et al., Lactobacillus rhamnosus GG supplementation for preventing respiratory infections in children: a meta-analysis of randomized, placebo-controlled trials, Indian Pediatr. 50 (4) (2013) 377–381.
[10] N. Pahumunto, G. Dahlen, R. Teanpaisan, Evaluation of potential probiotic properties of Lactobacillus and Bacillus strains derived from various sources for their potential use in swine feeding, Probiotics and Antimicrobial Proteins 15 (2021) 479–490.

[11] S. Thananimit, N. Pahumunto, R. Teanpaisan, Characterization of short chain fatty acids produced by selected potential probiotic Lactobacillus strains, Biomolecules 12 (12) (2022).
[12] N. Pahumunto, Y. Duangnumsawang, R. Teanpaisan, Effects of potential probiotics on the expression of cytokines and human β-defensins in human gingival epithelial cells and in vivo efficacy in a dog model, Arch. Oral Biol. 142 (2022) 105513.
[13] R. Teanpaisan, S. Piwat, G. Dahlen, Inhibitory effect of oral Lactobacillus against oral pathogens, Lett. Appl. Microbiol. 53 (4) (2011) 452–459.
[14] A. Chukamnerd, et al., BacSeq: a user-friendly automated pipeline for whole-genome sequence analysis of bacterial genomes, Microorganisms 11 (7) (2023) 1769.
[15] A. Bankevich, et al., SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing, J. Comput. Biol. 19 (5) (2012) 455–477.
[16] T. Seemann, Prokka: rapid prokaryotic genome annotation, Bioinformatics 30 (14) (2014) 2068–2069.
[17] T.M. Lowe, P.P. Chan, tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes, Nucleic Acids Res. 44 (W1) (2016) W54–W57.
[18] K. Lagesen, et al., RNAmmer: consistent and rapid annotation of ribosomal RNA genes, Nucleic Acids Res. 35 (9) (2007) 3100–3108.
[19] K. Surachat, et al., Genomic insight into pediococcus acidilactici HN9, a potential probiotic strain isolated from the traditional Thai-style fermented beef nhang, Microorganisms 9 (1) (2020).
[20] I. Grissa, G. Vergnaud, C. Pourcel, CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats, Nucleic Acids Res. 35 (2007) W52–W57.
[21] P. Stothard, D.S. Wishart, Circular genome visualization and exploration using CGView, Bioinformatics 21 (4) (2005) 537–539.
[22] S. Cosentino, et al., PathogenFinder - distinguishing friend from foe using bacterial whole genome sequence data, PLoS One 8 (10) (2013).
[23] V. Bortolaia, et al., ResFinder 4.0 for predictions of phenotypes from genotypes, J. Antimicrob. Chemother. 75 (12) (2020) 3491–3500.
[24] B.P. Alcock, et al., Card 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database, Nucleic Acids Res. 48 (D1) (2020) D517–D525.
[25] B. Liu, et al., Vfdb 2019: a comparative pathogenomic platform with an interactive web interface, Nucleic Acids Res. 47 (D1) (2019) D687–D692.
[26] A. Goel, P.M. Halami, J.P. Tamang, Genome analysis of Lactobacillus plantarum isolated from some Indian fermented foods for bacteriocin production and probiotic marker genes, Front. Microbiol. 11 (2020).
[27] P. Jarocki, et al., Complete genome sequence of Lactobacillus rhamnosus Pen, a probiotic component of a medicine used in prevention of antibiotic-associated diarrhoea in children, Gut Pathog. 10 (2018) 5.
[28] M.A. Nadkarni, et al., Comparative genome analysis of Lactobacillus rhamnosus clinical isolates from initial stages of dental pulp infection: identification of a new exopolysaccharide cluster, PLoS One 9 (3) (2014) e90643.
[29] S. Kandasamy, et al., Probiogenomic in-silico analysis and safety assessment of lactiplantibacillus plantarum DJF10 strain isolated from Korean raw milk, Int. J. Mol. Sci. 23 (22) (2022).
[30] A. de Jong, et al., BAGEL2: mining for bacteriocins in genomic data, Nucleic Acids Res. 38 (2010) W647–W651.
[31] A.J. van Heel, et al., BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins, Nucleic Acids Res. 46 (W1) (2018) W278–W281.
[32] Blin K., et al., antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation, Nucleic Acids Res. 51 (W1) (2023) W46–W50.
[33] E.V. Starikova, et al., Phigaro: high-throughput prophage sequence annotation, Bioinformatics 36 (12) (2020) 3882–3884.
[34] M.H.K. Johansson, et al., Detection of mobile genetic elements associated with antibiotic resistance in Salmonella enterica using a newly developed web tool: MobileElementFinder, J. Antimicrob. Chemother. 76 (1) (2021) 101–109.
[35] C. Bertelli, et al., IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets, Nucleic Acids Res. 45 (W1) (2017) W30–W35.
[36] M. Kankainen, et al., Comparative genomic analysis of Lactobacillus rhamnosus GG reveals pili containing a human- mucus binding protein, Proc. Natl. Acad. Sci. U.S.A. 106 (40) (2009) 17193–17198.
[37] M.I. Petrova, et al., Comparative genomic and phenotypic analysis of the vaginal probiotic Lactobacillus rhamnosus GR-1, Front. Microbiol. 9 (2018) 1278.
[38] A.J. Page, et al., Roary: rapid large-scale prokaryote pan genome analysis, Bioinformatics 31 (22) (2015) 3691–3693.
[39] R.C. Edgar, MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res. 32 (5) (2004) 1792–1797.
[40] S. Kumar, , et al.X. Mega, Molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (6) (2018) 1547–1549.
[41] E.V. Koonin, Y.I. Wolf, Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world, Nucleic Acids Res. 36 (21) (2008) 6688–6719.
[42] M.G. Langille, F.S. Brinkman, Bioinformatic detection of horizontally transferred DNA in bacterial genomes, F1000 Biol Rep 1 (2009) 25.
[43] S. Cosentino, et al., PathogenFinder–distinguishing friend from foe using bacterial whole genome sequence data, PLoS One 8 (10) (2013) e77302.
[44] P. Horvath, R. Barrangou, CRISPR/Cas, the immune system of bacteria and archaea, Science 327 (5962) (2010) 167–170.
[45] L.A. Marraffini, E.J. Sontheimer, CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA, Science 322 (5909) (2008) 1843–1845.
[46] H. Yli-Knuuttila, et al., Colonization of Lactobacillus rhamnosus GG in the oral cavity, Oral Microbiol. Immunol. 21 (2) (2006) 129–131.
[47] F.P. Douillard, et al., Comparative genomic and functional analysis of 100 Lactobacillus rhamnosus strains and their comparison with strain GG, PLoS Genet. 9 (8) (2013) e1003683.
[48] P. Varmanen, K. Savijoki, Responses of lactic acid bacteria to heat stress, in: E. Tsakalidou, K. Papadimitriou (Eds.), Stress Responses of Lactic Acid Bacteria, Springer US, Boston, MA, 2011, pp. 55–66.
[49] A. Guidone, et al., Polymorphisms in stress response genes in Lactobacillus plantarum: implications for classification and heat stress response, Ann. Microbiol. 65 (1) (2015) 297–305.
[50] M. Kumari, et al., Vitamin B12 biofortification of soymilk through optimized fermentation with extracellular B12 producing Lactobacillus isolates of human fecal origin, Curr. Res. Food Sci. 4 (2021) 646–654.
[51] N. Westerik, et al., Lactobacillus rhamnosus probiotic food as a tool for empowerment across the value chain in africa, Front. Microbiol. 9 (2018) 1501.
[52] J. Cheng, et al., Lactobacillus rhamnosus GG promotes intestinal vitamin D absorption by upregulating vitamin D transporters in senile osteoporosis, Calcif. Tissue Int. 111 (2) (2022) 162–170.
[53] R. Dimitrijević, et al., The identification of a low molecular mass bacteriocin, rhamnosin A, produced by Lactobacillus rhamnosus strain 68, J. Appl. Microbiol. 107 (6) (2009) 2108–2115.
[54] L.C. Oliveira, et al., In silico prediction, in vitro antibacterial spectrum, and physicochemical properties of a putative bacteriocin produced by Lactobacillus rhamnosus strain L156.4, Front. Microbiol. 8 (2017) 876.
[55] G.N. Tenea, C. Ortega, Genome characterization of lactiplantibacillus plantarum strain UTNGt2 originated from theobroma grandiflorum (white cacao) of Ecuadorian amazon: antimicrobial peptides from safety to potential applications, Antibiotics (Basel) 10 (4) (2021).
[56] S. Yeo, et al., Complete genome sequence of a potential probiotic Lacticaseibacillus rhamnosus strain LDTM 7511, isolated from Korean infant feces, Korean J. Microbiol. 58 (2) (2022) 85–87.
[57] U. Dobrindt, et al., Bacterial genome plasticity and its impact on adaptation during persistent infection, Int J Med Microbiol 300 (6) (2010) 363–366.
[58] P.M. Bennett, Genome plasticity: insertion sequence elements, transposons and integrons, and DNA rearrangement, Methods Mol. Biol. 266 (2004) 71–113.
[59] W. Li, A. Wang, Genomic islands mediate environmental adaptation and the spread of antibiotic resistance in multiresistant Enterococci - evidence from genomic sequences, BMC Microbiol. 21 (1) (2021) 55.
[60] J. Hacker, E. Carniel, Ecological fitness, genomic islands and bacterial pathogenicity. A Darwinian view of the evolution of microbes, EMBO Rep. 2 (5) (2001) 376–381.
[61] A.U. Happel, et al., In silico characterisation of putative prophages in lactobacillaceae used in probiotics for vaginal health, Microorganisms 10 (2) (2022).
[62] E. Durmaz, et al., Genome sequence and characteristics of Lrm1, a prophage from industrial Lactobacillus rhamnosus strain M1, Appl. Environ. Microbiol. 74 (15) (2008) 4601–4609.
[63] S. Casjens, Prophages and bacterial genomics: what have we learned so far? Mol. Microbiol. 49 (2) (2003) 277–300.

[64] Y.C. Kuo, et al., Characterization of putative class II bacteriocins identified from a non-bacteriocin-producing strain Lactobacillus casei ATCC 334, Appl. Microbiol. Biotechnol. 97 (1) (2013) 237–246.
[65] K. Surachat, et al., In silico analysis of protein toxin and bacteriocins from Lactobacillus paracasei SD1 genome and available online databases, PLoS One 12 (8) (2017).
[66] D.E. Kiousi, et al., Genetic and phenotypic assessment of the antimicrobial activity of three potential probiotic lactobacilli against human enteropathogenic bacteria, Front. Cell. Infect. Microbiol. 13 (2023) 1127256.