RESEARCH ARTICLE

In silico analysis of protein toxin and bacteriocins from Lactobacillus paracasei SD1 genome and available online databases

Komwit Surachat¹,³, Unitsa Sangket¹,², Panchalika Deachamag¹,², Wilaiwan Chotigeat¹,²*¹

Department of Molecular Biotechnology and Bioinformatics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand, ²Center for Genomics and Bioinformatics Research, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand, ³Information and Communication Technology Programme, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

* wilaiwan58@hotmail.com

Abstract

Lactobacillus paracasei SD1 is a potential probiotic strain due to its ability to survive several conditions in human dental cavities. To ascertain its safety for human use, we therefore performed a comprehensive bioinformatics analysis and characterization of the bacterial protein toxins produced by this strain. We report the complete genome of Lactobacillus paracasei SD1 and its comparison to other Lactobacillus genomes. Additionally, we identify and analyze its protein toxins and antimicrobial proteins using reliable online database resources and establish its phylogenetic relationship with other bacterial genomes. Our investigation suggests that this strain is safe for human use and contains several bacteriocins that confer health benefits to the host. An in silico analysis of protein-protein interactions between the target bacteriocins and the microbial proteins gtfB and luxS of Streptococcus mutans was performed and is discussed here.

Introduction

The term 'probiotics' has been defined by a joint consultation of experts from the Food and Agricultural Organization of the United Nations and World Health Organization as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” [1]. They have diverse key functions, such as activities against other microorganisms [2], modulation of the host immune system [3], and the prevention of carcinogenic processes [4, 5], allergic diseases [6] and oral diseases [7–9]. The use of probiotics has been proposed for the maintenance of microbial homeostasis in the oral cavity, gastrointestinal tract and vagina. In addition, especially in oral health applications, probiotics could beneficially alter the biofilm microbial composition [10] or stimulate the host immune response [11]. One of the organisms with great potential to inhibit the growth and persistence of detrimental resident bacteria in specific oral areas is Lactobacilli sp. [12–14].
For administration as human oral probiotics, various strains and species of Lactobacillus have been studied. Lactobacillus rhamnosus GG [15], for example, has been investigated in vitro and tested in vivo in the oral cavity. However, the results reported that permanent colonization of the oral cavity was not consistently achieved. It seems to occur only in some individual patients. Another study [16] investigated the probiotic activity of L. reuteri DSM 17938, L. acidophilus DDS-1, L. rhamnosus ATCC 53103, and L. paracasei B21060 and characterized their benefits in terms of their reduction of surface tension and emulsifying ability. Against test pathogens Streptococcus mutans ATCC 25175 and Streptococcus oralis ATCC 9811, Lactobacilli significantly inhibited adhesion and biofilm formation on titanium surfaces. Although researchers have performed and proposed many studies about the oral origin of antimicrobial proteins or bacteriocins produced by Lactobacilli, more knowledge about their safety is still required.

Recently, Rawee et al. [17–19] isolated L. paracasei SD1 from the human oral cavity, and the isolate significantly reduced populations of oral S. mutans, perhaps discovering a new probiotic that can control bacterial communities in the human mouth. The results of the in vitro research reported a protein with an approximate molecular weight of 25 kDa that exhibited a broad spectrum against oral pathogens. This result showed the possible application of this bacteriocin in the prevention and treatment of oral diseases. However, even though the antibacterial activity produced by L. paracasei SD1 may have already been explored and characterized, the whole genome sequence and related knowledge must be thoroughly investigated before it may be considered safe for human applications.

In this paper, we will present and discuss an in silico analysis of bacterial protein toxins and bacteriocins from L. paracasei SD1. This study aims to sequence and assemble the whole genome, analyze all possible protein toxins, and identify all antimicrobial proteins or bacteriocins in the genome and plasmids. The workflows for the identification of protein toxins and bacteriocins were put together from available online databases, and these identifications are reported here. Many interesting bacteriocins were identified in the genome and plasmid of SD1: Carnocin-CP52, Enterocin Xβ, Gassericin A, LSEI_2163 and LSEI_2386. Additionally, the list of identified toxins in the SD1 genome was reported, and the safety of their use in dairy products is discussed. To show the binding potential and discuss the mechanism inhibiting the growth of S. mutans, we simulated in silico protein-protein interactions between the bacteriocins and the gtfB and luxS membrane proteins of S. mutans, which are involved in biofilm formation. This study could confirm the safety and benefits of L. paracasei SD1 in probiotic products due to its inhibition of other bacterial strains.

Materials and methods

Isolation and purification of genomic DNA

L. paracasei SD1 was donated by Prof. Dr. Rawee Teanpaisan of the Common Oral Diseases and Epidemiology Research Center, Faculty of Dentistry, Prince of Songkla University. DNA extraction was performed with DNeasy extraction kit (QIAGEN, Hilden, Germany) following the manufacturer’s instructions. Briefly, the bacterial cell pellet was resuspended in 180 μL of enzymatic lysis buffer and incubated for 30 minutes at 37˚C and 25 μL of proteinase K 200 μl Buffer AL was then added and mixed before incubating at 56˚C for 30 min. Then, 200 μL of ethanol was added to the DNA sample and centrifuged through the DNeasy Mini spin column at 610 g for 1 minute. After that, DNA was washed with 500 μL of Buffer AW2 and then eluted with buffer AE. Finally, the DNA concentration in the eluate was measured by a spectrophotometer at 260 nm. The ratio of the absorbance at 260 nm and 280 nm (A260/A280) provided an estimate of the purity of DNA by agarose gel electrophoresis.
**Genome sequencing**

The *L. paracasei* SD1 genome (Accession no. SRP091927) was sequenced using PacBio P6C4 chemistry on a PacBio RSII sequencer (Pacific Biosciences, California, USA). One single-molecule real-time (SMRT) cell was used for sequencing, yielding 150,292 subreads with an average read length of approximately 5 kbp. The hierarchical genome assembly process (HGAP) method based on the SMRT Analysis package v2.3.0 in the SMRT Portal was used for de novo assembly. The following assembly parameters were set: minimum subread length at 500 bp, minimum read score at 0.80 and length cutoff at 5,000 bp. There were 74,620,028 pre-assembled bases with 22,624 pre-assembled reads. As a result, the assembly yielded six contigs with the largest contig being approximately 2.84 Mbp. The Gepard visualization tool [20] was used to evaluate the assembly by drawing the genome dot plot. Finally, Circlator [21], a tool for automated circularization of genome assemblies, was used to concatenate all the contigs into one closed circular chromosome and two circular plasmids.

**Genome and plasmid annotation**

Initial automated gene calling was performed using Glimmer 3 [22] and Genemark [23–25]. Functional annotation was achieved using RAST (Rapid Annotation using Subsystem Technology) [26, 27], tRNA was predicted by tRNAscan-SE 1.21 [28–31] and rRNA genes were predicted by RNAmmer 1.2. The CDS, genes and related regions were annotated by mapping the plasmids to a plasmid database, which was downloaded from NCBI on 1-07-2016. All the predicted proteins were searched (BLASTP) against the NCBI non-redundant (nr) protein database. Prophage regions were predicted using the PHAge Search Tool (PHAST) web server [32], and regions of clustered regularly interspaced short palindromic repeats (CRISPR) were searched using the CRISPRFinder server [33].

**Genome comparisons**

*L. paracasei* SD1 was first searched with the microbial nucleotide BLAST to find the closest organism for comparison. *L. paracasei* JCM 8130 (NC_008526), *L. casei* ATCC 334 (NC_008526) and *L. paracasei* KL1 (NZ_CP013921) were the top three blast hits with approximately 99% identity and very significant E-values. Based on the BLASTX comparison, the similarity of SD1 with its three closest relatives was analyzed and visualized using the CGView Server [34] by setting the expect value cutoff to 0.00001 and the identity cutoff to 30%.

**Phylogenetic analysis**

16S rRNA sequences (approximately 1,540 bp) were retrieved from the following 32 species: *L. johnsonii* NCC 533, *L. johnsonii* FI9785, *L. johnsonii* N6.2, *L. johnsonii* BS15, *L. acidophilus* La-14, *L. acidophilus* NCFM, *L. acidophilus* FSI4, *L. reuteri* DSM 20016, *L. reuteri* SD2112, *L. reuteri* ZLR003, *L. reuteri* I49, *L. plantarum* DF, *L. plantarum* B21, *L. plantarum* WCFS1, *L. plantarum* KP, *L. salivarius* st. Ren, *L. salivarius* JCM 1046, *L. salivarius* CECT 5713, *L. salivarius* UCC118, *L. paracasei* KL1, *L. paracasei* JCM 8130, *L. paracasei* N115, *L. paracasei* L9, *L. casei* Zhang, *L. casei* LOCK919, *L. casei* BD-II, *L. casei* W56, *L. rhamnosus* LOCK908, *L. rhamnosus* BFE5264, *L. rhamnosus* ATCC 8530, *L. rhamnosus* ATCC 53103 and *L. lactis* KF147. A multiple alignment of the 16S rRNA nucleotides was generated using MUSCLE [35]. The phylogenetic tree was constructed by Geneious 9.1.2 [36, 37] using the neighbor-joining method with a bootstrap value of 1,000.
Genome and plasmid visualization
The circular representation of the chromosome of L. paracasei SD1 was produced using the CGView Server V 1.0 [34]. The subsystem mapping was produced by RAST version 2.0 [26, 27]. In addition, the circular representation of the plasmids was drawn using Geneious 9.1.2 [36, 37].

Protein toxin identification
The workflow was constructed with two main processes: a preliminary identification and the local blast and sequence alignment. The preliminary identification was implemented to search related genes and proteins using BLASTN in the VFDB database [38–41] and BLASTX in the DBETH database [42], respectively. The preliminary result list was generated individually. We then further analyzed and investigated the results by searching the NCBI and the UniProt databases and gathering a set of similar genes and proteins in Lactobacilli organisms to create our own local databases. Using BLASTN with the NCBI nucleotide sequence and BLASTX with the UniProt protein sequence, we set the organism to Lactobacillus and E-value to $10^{-4}$ to create our own local databases. Finally, the result lists were compiled by searching the preliminary results with the created databases and aligning sequences individually with the references. The final result was the confirmed list of genes and protein toxins in L. paracasei SD1. The workflow of protein toxin identification is given in S1 Fig.

Antibiotic-resistant protein identification
The presence of antibiotic-resistance genes in L. paracasei SD1 was analyzed by using hmmscan to the proteomes of the chromosome and plasmids against the Resfams database [43]. The possibility of horizontal transfer of antibiotic-resistance genes to other commensals was then identified by searching against the ICEberg database [44].

Bacteriocin identification
The first step of the bacteriocin identification workflow was creating a BACTERIOCINS local database by merging the BAGEL (class I, II and III) [45] and BACTIBASE databases [46, 47]. The nucleotide sequence of L. paracasei SD1 was first screened with the BACTERIOCINS database using BLASTX. The preliminary screening result was then generated and used as the target of a search for similar protein sequences from Lactobacilli in the NCBI and UniProt databases. The local database was then created after removing data redundancy in the database. Finally, the preliminary result from the first blast was then searched again against the newly created database using BLASTX with the E-value set to $10^{-4}$ and an identity cutoff at 30%. The final result was then aligned to find the conserved sequence and confirm it as a bacteriocin in L. paracasei SD1. The workflow is given in Fig 1.

Protein-protein interaction analysis
The 3D structure of three bacteriocins, namely, LSEI_2163, LSEI_2386, and Gassericin A of L. paracasei SD1, were predicted using the RaptorX server [48–51], I-TASSER server [52–56] and QUARK server [57]. The predicted models were then evaluated using RAMPAGE [58] and PROCHECK [59]. The predicted model of LSEI_2163 came from the RaptorX server, whereas the other two models were from the I-TASSER server due to their higher scores and the absence of residues in allowed regions and outlier regions. In addition, the sequences of gtfB and luxS of S. mutans were obtained from the UniProt database with primary accession numbers Q8DVK8 and P08987, respectively.
**Fig 1. The bacteriocin identification workflows.** There were four processes in the workflow: database preparation, preliminary analysis, local database preparation, and bacteriocin identification.

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The 3D structures of luxS and gtfB were then modeled by SWISS-MODEL [60–64]. The templates for luxS and gtfB were S-ribosylhomocysteine lyase from S. mutans strain ATCC 700610/UA159 (SMTL id: 4xch.1.A, X-RAY DIFFRACTION 2.20 Å, residue 12–157) with 82.17% sequence identity and the crystal structure of Glucosyltransferase-I from S. mutans strain ATCC 700610/UA159 (SMTL id: 3tto.1.A, X-RAY DIFFRACTION 3.30 Å, residue 159–1058) with 48.95% sequence identity, respectively. Then, protein-protein docking was performed by the PIPER program [65] on the ClusPro 2.0 server [66–68]. The model with the largest cluster and the lowest docking score of the balance mode was selected. Afterwards, the selected model was elucidated by the PyMOL program [69]. The determined values included scores for balanced, electrostatic, hydrophobic, and Van der Waals interactions.

Results and discussion

Genome features of L. paracasei SD1

Whole-genome sequencing was processed with the PacBio single-molecule real-time (SMRT) sequencing system to determine the genome sequence of L. paracasei SD1. De novo assembly generated five contigs using the HGAP v3.0 from PacBio reads obtained using P6C4 chemistry. We then further assembled and verified the finished single complete genome. The genome of L. paracasei SD1 composes of one circular chromosome (2,995,875 bp) and two circular plasmids designated, as pSD1-1 (11,281 bp) and pSD1-2 (10,902 bp). The general genomic features and information are given in Table 1.

Four intact prophages were predicted in chromosome ϕSD1-1 (681–709 kb), ϕSD1-2 (822–864 kb), ϕSD1-3 (1,828–1,876 kb) and ϕSD1-4 (1,939–1,993 kb). No prophage was identified in the plasmids. Most of the identified prophage CDS were hypothetical proteins. The prophages are known as the inactive forms of the bacteriophage and are commonly found in Lactobacillus organisms. In addition, we also explored some Lactobacillus organisms and identified prophage regions to compare with L. paracasei SD1. We investigated the genomes of L. paracasei JCM 8130, L. paracasei strain KL1, L. casei ATCC 334, L. paracasei 8700:2, L. casei W56, L. casei BD-II chromosome, L. casei LOCK919, L. brevis ATCC 367, L. fermentum IFO 3956, L. plantarum WCSF1, L. reuteri DSM 20016, L. rhamnosus GG, and L. salivarius UCC118. As a result, we identified predicted prophages in every target genome. The prophage contents of Lactobacilli were similar, and they shared many CDS in the chromosome.

Table 1. Genome and plasmid features and information for Lactobacillus paracasei SD1.

|                        | SD1 genome | pSD1-1 | pSD1-2 |
|------------------------|------------|--------|--------|
| Sequencing technology  | PacBio P6C4|        |        |
| BioProject number      | PRJNA349248|        |        |
| BioSample number       | SAMN05929041|       |        |
| Submission number      | SRP091927|        |        |
| Size (in bp)           | 2,995,875  | 11,281 | 10,902 |
| GC content (%)         | 46.6       | 42.5   | 40.2   |
| CDS                    | 2984       | 15     | 10     |
| Hypothetical protein   | 962        | 11     | 8      |
| Subsystems             | 338        |        | -      |
| tRNA                   | 61         |        | -      |
| rRNA                   | 15         |        | -      |

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Additionally, we used the IslandViewer 3 application [70] to identify genomic islands (GIs) in *L. paracasei* SD1. Seventeen GIs were predicted with an average length of 9.2 kbp, corresponding to a total sequence 156 kbp or 5.22% of the genome. This externally acquired DNA percentage is lower than that of the reference genome, *L. casei* ATCC 334, with 8.76%, but it is higher than those of other members of the *L. paracasei* group, which ranged from 1.6% in *L. paracasei* JCM 8130 to 0.84% in *L. paracasei* KL1. Multiple mobile genetic elements (MGEs), including prophages and transposons, were identified in the GI elements. Most of the MGEs found in the genome contained similarities to integrative and conjugative elements (ICEs), which can be integrated into or excised from the host chromosome. Normally, ICEs provide increased adaptation to the host, through genetic improvements to phage resistance and metal transport, for instance. Genes similar to transmembrane proteins and phage-related proteins were identified in the genome. We suggest that these genes are related to increasing the incorporation and stability of this bacterial strain in the human oral cavity, which thus improves its local fitness.

In addition, no CRISPR loci were identified in *L. paracasei* SD1. NCBI microbial nucleotide BLAST was used to evaluate its similarity with other microbial genomes. The *L. paracasei* SD1 genome was searched against all completed genomes in the NCBI databases. The closest microbial organism was *L. paracasei* JCM 8130, followed by *L. casei* ATCC 334 and *L. paracasei* KL1. The identity of the top three closest genomes was around 99%. The similarity based on BLASTX comparison with its three close relatives was analyzed and visualized as shown in Fig 2. The red boxes highlight several unique regions in *L. paracasei* SD1 (region I: from \( \approx 28^\circ \) to \( 32^\circ \), region II: from \( \approx 88^\circ \) to \( 92^\circ \), region III: from \( \approx 224^\circ \) to \( 227^\circ \) and region IV: from \( \approx 308^\circ \) to \( 312^\circ \)). Several CDS, including transposase proteins, transcriptional regulators, putative membrane proteins, FPXTG cell wall anchor family proteins, and alpha-galactosidase, were inserted in those regions (S1 Table), which may have affected the strain’s adaptation to specific ecological niches during its evolution.

Orthologous proteins among the closest strains of *L. paracasei* SD1 were obtained using the reciprocal all versus all BLAST. The analysis revealed that 2427 proteins formed the core set of proteins among the three strains. Orthology charts were plotted from the orthologous proteins as shown in Fig 3. *L. paracasei* SD1 shared 2,683 orthologous pairs with *L. paracasei* JCM 8130 and 2,453 with *L. paracasei* KL1. The remaining proteins that contained no orthologous pairs were then extracted from the proteomes of *L. paracasei* SD1 (275 proteins) and subjected to BLASTP against the NR database. Out of 275 proteins in *L. paracasei* SD1, only eighteen (37–54 residues) did not match any sequence in the database and 168 proteins were annotated as hypothetical proteins. The rest of the proteins had homology to *L. paracasei* and *L. casei* proteins at > 85% identity and < 10\(^{-5}\) E-value.

**Plasmid features of *L. paracasei* SD1**

Two circular plasmids, pSD1-1 (11,281 bp) and pSD1-2 (10,902 bp) were identified during the assembly process. General information on pSD1-1 and pSD1-2 are given in Table 1. The sizes of these two plasmids are slightly different. The first plasmid, pSD1-1, is comprised of various regions from different species/subspecies of *L. paracasei*, *L. casei* and *L. gallinarum*, which indicates the evolution among them. There were 15 CDS in this plasmid, and 8 of them were annotated as hypothetical proteins. The other CDS comprised 1 ABC transporter, 1 cell division protein FtsX, 2 mobilization proteins, 2 plasmid replication proteins (RepB), and 1 Gas-sercin A. Interestingly, Mob plasmid mobilization proteins were identified in this plasmid and are probably required for relaxation complex formation and plasmid mobilization by conjugative plasmids. Another fascinating characteristic was the presence of a bacteriocin gene,
Fig 2. (A) Circular representation of the SD1 chromosome and (B) functional distribution of the SD1 coding genes based on SEED subsystems. (A) The outermost circle and the second circle show the positions of the CDS in clockwise and counterclockwise directions, respectively. Protein-coding genes, tRNA genes and rRNA genes are highlighted in different colors. The third, fourth, and fifth circles from the outside represent BLASTX comparisons with L. paracasei JCM 8130, L. casei ATCC 334 and L. paracasei KL1, respectively. The innermost and second inner circle
Gassericin A, that contains antibacterial activity against a number of gram-positive food-borne pathogenic bacteria [71]. This mechanism may play an important role in inhibiting other bacterial activities in human.

The other plasmid, pSD1-2, shared many proteins with various plasmids and genomes of the bacteriocin-producing strain of \textit{Lactobacilli}, such as plasmid pLBPC-2 of \textit{L. paracasei} subsp. paracasei JCM 8130, the plasmid unnamed1 of \textit{L. paracasei} strain CAUH35, plasmid pW56 of \textit{L. casei} W56, and plasmid pBD-II of \textit{L. casei} BD-II. These strains also produce many

![Venn diagram](https://doi.org/10.1371/journal.pone.0183548.g003)

**Fig 3. Core, dispensable and unique sets of proteins.** Venn diagram of ortholog group distribution in \textit{L. paracasei} SD1, \textit{L. paracasei} JCM 8130, and \textit{L. paracasei} KL1.
bacteriocins, but there was no sign of antimicrobial proteins in these plasmids similar to pSD1-2 of SD1. The content of pSD1-2 comprised 10 CDS, which were annotated to 4 hypothetical proteins, 1 serine protease, peptidoglycan-binding protein, 2 RepB, 1 SM11/KNR4 family protein, and 1 protein that was not found in any of the available databases. In the case of SM11/KNR4, this protein is found in many species of Lactobacillus, Streptococcus, Geobacillus, Listeria, Bacillus, and others. Originally, SM11/KNR4 was found in a yeast cell wall. It plays a regulatory role in chitin deposition and cell wall assembly, and it connects to other pathways for cell proliferation. We can infer that SM11/KNR4 may have transferred during the evolution process while remaining functional in the plasmid of this bacterial strain. The features of both plasmids, pSD1-1 and pSD1-2, are illustrated in S2 Fig.

16S rRNA phylogenetic analysis of L. paracasei SD1 and other sequenced Lactobacilli

16S rRNA sequences (approximately 1,540 bp) were collected from Lactobacilli genomes (3 L. acidophilus, 4 L. casei, 4 L. johnsonii, 4 L. paracasei, 4 L. plantarum, 4 L. reuteri, 4 L. rhamnosus, 4 L. salivarius, and 1 Lactococcus lactis). There are many species of Lactobacilli that have more than one copy of ribosomal RNA in their genome; therefore, we used representative consensus sequences [72, 73] from multiple sequence alignment. Then, we constructed the phylogenetic relationships between L. paracasei SD1 and the others based on thirty-three 16S rRNA sequences, setting L. lactis KF147 as the outgroup. Additionally, we also identified the amount of bacteriocins produced by each strain in the chromosome using the BAGEL database. The result in Fig 4 shows that L. paracasei SD1 was placed in the casei group, which formed a distinct clade from L. rhamnosus. SD1 produced 4 bacteriocins in the genome, which was almost the same amount as the others in this clade. In addition, the produced bacteriocins shared among this group were the same protein, i.e., LSEI_2163, LSEI_2386 and Enterocin Xβ. This result could reveal how SD1 is phylogenetically close to the other members of this group.

Protein toxin report

There were 9 different protein toxins from 15 locations in the preliminary screening results from blasting the DBETH database [42] and just 6 records containing E-values less than 0.0001. We eliminated some results that contained E-values higher than 1e-4. The remaining records were 2 Hemolysin (1 from Vibrio cholerae MO10 and 1 from Vibrio cholerae CT 5369-93), 2 Hemolysin-III (1 from Bacillus cereus and 1 from Clostridium tetani), lepB and Phospholipase D. Thus, we further investigated the preliminary results by gathering information about the identified protein toxins in Lactobacilli from NCBI and UniProt and creating a local database. The blast results against the local databases confirmed that Phospholipase D was not found in any part of L. paracasei SD1. However, we identified a protein toxin in the Hemolysin family with a very low E-value and 100 percent identity with Hemolysin and Hemolysin-III in 2 different locations. These two proteins are also commonly found in many close organisms, such as L. casei, L. paracasei, L. rhamnosus, L. zeae, and L. saniviri. Unsurprisingly, there are many reports about Hemolysin and its safety in Lactobacilli [74, 75]. These reports suggested that Lactobacilli can be included in probiotic products without any danger to the host organism [1, 74–77].

The signal peptidase lepB was also investigated using the local database. The results contained very low E-values and very high percent identity (over 99%). This points to the presence of only three protein toxins in L. paracasei SD1, as shown in Table 2. The complete sequences of the three identified toxins are also given in S2 Table. We also searched the bacterial gene toxin in the VFDB database. No significant similarity was found with any of the genes.
Fig 4. Phylogenetic tree based on the neighbor-joining method of 16S rRNA gene sequences. The analysis involved 34 sequences of *Lactobacillus* species. The sequences were retrieved from 3 *L. acidophilus*, 4 *L. casei*, 4 *L. johnsonii*, 4 *L. paracasei*, 4 *L. plantarum*, 4 *L. reuteri*, 4 *L. rhamnosus*, 4 *L. salivarius*, and 1 *Lactococcus lactis*. *L. lactis* KF147 was set as the outgroup. The tree was created using a neighbor-joining method and numbers at branch points are bootstrap values (based on 1000 samplings expressed in percentages). The scale bar represents an evolutionary distance. The tree has been arbitrarily rooted. The number of bacteriocins produced by each strain is shown in parentheses.

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Table 2. Protein toxins identified in the SD1 genome and plasmid using BLASTX against DBETH database.

| Protein Name          | Bit-Score | Identity | Length | Mismatches | E-value | Location       | Frame |
|-----------------------|-----------|----------|--------|------------|---------|----------------|-------|
| Hemolysin-III         | 118.24    | 35.29    | 204    | 132        | 8.00E-28 | 1,386,627–1,387,238 | -2    |
| Hemolysin             | 77.41     | 23.4     | 265    | 184        | 5.00E-14 | 1,773,619–1,774,407 | -2    |
| lepB                  | 58.92     | 25.73    | 206    | 95         | 5.00E-08 | 252,436–252,933    | -1    |

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Hemolysis is a common virulence factor in microorganisms. It can facilitate iron availability and cause anemia and edema in the host [76]. These microorganisms need iron as a cofactor for several enzymes [78]. *Lactobacilli* can grow normally without iron, which is an ecological advantage in the natural environment, where they compete with pathogenic bacteria. That advantage could imply that the Hemolysin protein family found in *Lactobacilli* does not cause the lysis of human erythrocytes [77, 79, 80].

**Antibiotics-resistant protein report**

Presently, antibiotic-resistance is a major problem in treating bacterial infections. Antibiotic resistance can naturally occur or can be achieved by the overuse and misuse of antibiotics. Bacteria or other microbes can develop resistance to antibiotics by mutating existing genes or by acquiring new genes from other strains or species by genetic mechanisms, such as horizontal gene transfer through plasmids or transposons. Several species of *Lactobacillus* have been commercially used as probiotics. Thus, a safety concern due to the possibility of transferring antibiotic resistance via their plasmids or transposons should be identified and discussed.

We identified the presence of antibiotics-resistant proteins in both the chromosome and plasmids of *L. paracasei* SD1. The genome of SD1 was then subjected to hmmscan against the Resfams database [43]. There was no sign of the presence of any antibiotic-resistant proteins in plasmid pSD1-2. However, 3 Resfams domains were identified in plasmid pSD1-1. These domains had the same antibiotic mechanism, which is ABC transporters. ABC transporters are present in all cells of all organisms and use ATP binding energy to transport substrates across cell membranes. In the chromosome, 80 Resfams domains were identified, which can be classified by their antibiotic mechanisms into 14 groups (S3 Table). Beta-Lactamase, glycopeptide resistance and gene modulating resistance were the top three groups found in SD1. We then analyzed the possibility of horizontal transfer of the identified proteins to other commensals by searching the ICEberg database [44]. ICEberg is a database for integrative and conjugative elements (ICEs) found in both gram-positive and gram-negative bacteria. ICEs are self-transmissible mobile genetic elements that can be integrated into or excised from the host chromosome. Thus, they can promote their own mobilization and horizontal transfer of virulence determinants, antibiotic-resistance genes and other bacterial traits. We then used plasmid pSD1-1 as a search target in the ICEberg database using the BLASTN program by with the expect value cutoff set to 1e-4. No matches came from plasmid pSD1-1. Thus, we suggested that the transfer of any antibiotic-resistance genes from the plasmid should not occur at this stage because its lack of ICEs and relevant elements. In addition, no large mobile genetic elements were identified in the chromosome. However, antibiotic resistance may be transferred via plasmid transfer. There were many short fragments matches to the database with small lengths (between 47–96 bp). Their percentage of query sequence coverage was too small compared to the target sequences. Consequently, we suggested that SD1 could not transfer any antibiotic-resistance genes to other commensals.

**Bacteriocin report**

We identified four different bacteriocins in five different locations in the genome and four different bacteriocins in the same location as plasmid pSD1-1 as shown in Table 3. After further investigation with the locally created bacteriocin database of *Lactobacilli*, we suggest that the *L. paracasei* SD1 genome has a number of bacteriocins: LSEI_2163, LSEI_2386, Carnocin-CP52 immunity protein and Enterocin Xβ. Additionally, in the *L. paracasei* SD1 plasmid, we identified a bacteriocin of the Gassericin family. The sequences of all bacteriocins and their molecular weights are given in S4 Table.
Roughly, 30–90% of bacteria can produce at least one bacteriocin [81] which could imply that there are many undiscovered bacteriocins in both producing and non-producing strains of bacteria. Based on published knowledge, we identified at least five bacteriocins in this strain, as mentioned above. Two of them, LSEI_2163 and LSEI_2386, were putative pheromone peptides that have significant bacteriocin activity against several *Listeria* species [82], which could make this bacterium useful in medical applications. Enterocin Xβ is a class IIb bacteriocin that was originally found in *E. faecium* KU-B5 [83]. However, no research has reported Enterocin Xβ present in *Lactobacillus*. Only Enterocin A protein has been reported in the *Lactobacillus* genus according to study [84].

From our identification results (51% identity, 80.46% coverage, 23 bases out of 51 were mismatched, and an E-value approximately 4e-13), we could not exactly confirm the presence of Enterocin Xβ in the genome. We identified a related result with 100% identity for bacteriocin leader domain-containing protein, which is found in many strains of *Lactobacillus casei* and *paracasei*. However, we suggest that our proposed strain may contain bacteriocin-like proteins and still need to explore their mechanisms and antibacterial activity. In addition, we cannot definitely confirm that the Carnocin-CP52-like protein found in this genome was the same as the bacteriocin in the bacteriocin databases. It seemed to contain high confidence, with an of E-value approximately 3e-20, but its identity and coverage percentages were 32% and 98.20%, respectively. There were 74 mismatched bases out of 110.

Further investigation was then conducted with BAGEL3, a web-based bacteriocin mining tool. BAGEL3 combines direct and indirect mining by looking at context genes. Therefore, it can explore bacteriocin-encoding genes or other bacterial ribosomally synthesized and post-translationally-modified peptides (RiPPPs) in the target genetic data. The result from BAGEL3 showed that bacteriocins were identified in four regions of the chromosome, as shown in Fig 5A. Other biosynthetic genes were present in regions I, II and IV. In region III, only two bacteriocin genes were identified. Region I contained a 9,944-bp area of interest comprised of two genes. This cluster contained a glutamine amidotransferase domain and a putative class II bacteriocin gene (LSEI_2163). Region II was found to contain a 9,944-bp class II bacteriocin

### Table 3. Bacteriocins identified in the SD1 genome and plasmid by blasts against BACTIBASE and BAGEL databases.

| Protein Name                      | Bit-Score | Identity | Alignment Length | Mismatches | E-value   | Location | Frame |
|-----------------------------------|-----------|----------|------------------|------------|-----------|----------|-------|
| LSEI_2386                         | 95.9      | 100      | 45               | 0          | 6.00E-23 | 2,423,659| -1    |
|                                   |           |          |                  |            |           | 2,423,793|       |
| LSEI_2163                         | 84.34     | 100      | 39               | 0          | 6.00E-19 | 2,207,159| -3    |
|                                   |           |          |                  |            |           | 2,207,275|       |
| Carnocin-CP52 immunity protein    | 65.47     | 31.82    | 110              | 74         | 7.00E-12 | 2,432,966| -3    |
| Enterocin Xβ                      | 46.60     | 50.98    | 51               | 23         | 4.00e-13 | 2,428,795| -1    |
|                                   | 41.20     | 64       | 25               | 9          | 4.00E-04 | 2,431,600| -1    |
| Butyrivibriocin AR10              | 72.40     | 55.0     | 60               | 27         | 5.05E-18 | 5,932    | 6,111 |
| Pentocin                          | 59.31     | 40.7     | 81               | 44         | 4.10E-13 | 5,926    | 6,168 |
| Gassericin A                      | 49.68     | 37.8     | 74               | 42         | 5.41E-10 | 5,932    | 6,153 |
| Acidocin B                        | 48.13     | 50.0     | 44               | 22         | 2.06E-09 | 5,932    | 6,063 |

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Fig 5. Gene clusters of the SD1 chromosome and plasmid. (A) Region I-IV represent gene clusters identified in the chromosome. (B) Region P_I represents gene clusters identified in the plasmid pSD1-1.

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cluster containing five genes, including one immunity/transport, one transport and leader cleavage, one bacteriocin and two undefined associated sites. An immunity/transport site that was found in this cluster was significantly similar to the bacteriocin ABC transporter, plnH. A transport and leader cleavage site, Bacteriocin ABC exporter (Pep2E family), was identified in this cluster. This protein involves bacteriocin transmembrane transporter activity, ATPase activity and is coupled to transmembrane movement of substances. The other genes were comprised of a putative class II bacteriocin gene (LSEI_2386), a response regulator (plnD) and one protein belonging to transmembrane fragile-X-F protein domain. Region III contained only two class II bacteriocin genes, Carnocin-CP52 immunity protein and Enterocin Xβ. There no signs of any biosynthetic genes. With the lack of a bacteriocin-associated domain in the cluster, we can only suggest that this strain may contain some immunity-like proteins, which have been submitted in the UniProt and NCBI databases under many names: possibly putative carnobacteriocin-B2 immunity protein, bacteriocin immunity protein, prebacteriocin, and others.

Surprisingly, we did not find any bacteriocins in region IV using BLASTX against the bacteriocin database. In contrast, BAGEL3 can predict a bacteriocin from this cluster. There were two immunity/transport domains, including a Type II/IV secretion system protein and a Nod factor export ATP-binding protein I. A head to tail cyclized peptide identified in this region did not match any record in the database. The sequence was then used to BLASTP search the UniProt database. The hit result with 100 percent identity was a membrane protein that was reported in many organisms of the Lactobacillus species. This protein belongs to Stage II sporulation protein M (SpoIIM), which is necessary for the forespore inside the mother-cell to be properly internalized through the breakdown of peptidoglycans trapped between the membranes of the septum separating the forespore and the mother-cell. SpoIIM was originally reported in Bacillus subtilis, where it was responsible for localization to the septal membrane.

In addition to the bacteriocins identified in the genome, there was also 1 bacteriocin in plasmid pSD1-1, Gassericin A, as shown in Fig 5B. This cluster contained two immunity/transport domains including a YlqD protein and a Type II/IV secretion system protein, and one modification domain, NADH dehydrogenase. Gassericin A is a circular bacteriocin produced by lactic acid bacteria that was originally discovered in L. gasseri. Gassericin A inhibits the growth of a wild type of toxic S. aureus isolated from mastitis milk and breast organs [71, 85, 86]. Additionally, there was another submission of Gassericin A in the L. paracasei Lpp41 strain, which could be an important finding, since it can be a crucial tool in food preservation due to its high pH and temperature tolerance [85].

**Bacteriocin interaction**

Previous reports have shown that S. mutans and S. sobrinus were inhibited by L. paracasei SD1 [18, 19], but the mechanism is still unknown. In this *in silico* study, protein interactions between identified bacteriocins and the gtfB and luxS proteins of S. mutans were performed. Three bacteriocins from L. paracasei SD1, namely, Gassericin A, LSEI_2386 and LSEI_2163, were selected to be paired and docked to the target proteins because previous studies reported [82, 87–90] that those bacteriocins exhibited antimicrobial activity and inhibited several bacteria in vitro.

The reported mode of action of Gassericin A was to permeate the membrane and cause bacteriolysis [91]. However, the experiment performed by Kawai et al. [92] showed that bacteriocin concentrations were higher than what was required for antimicrobial activity in vivo. In addition, it has been a controversial issue as to whether bacteriocins require a receptor molecule or not. There was some evidence that the antimicrobial activity of class II bacteriocins was
the receptor mediated by recognition of specific proteins on the membrane of target cells [93]. According to a report [94], some species of *Lactobacillus* that may contain the Gassericin family have the ability to decrease the biofilm formation of clinically isolated *S. mutans*. We then perform an *in silico* survey of the binding any target protein on the membrane of *S. mutans*.

The binding of bacteriocin to gtfB and luxS is based on how bacteriocin can control other microorganisms with gtfB and luxS as examples. gtfB from *S. mutans* produces water-insoluble glucans that have the ability to adhere to smooth and aggregated bacterial cells and food debris; thus, dental plaque occurs. We showed that the binding between bacteriocins and gtfB supports the prevention of gtfB to induce plaque. In addition, luxS is a S-ribosyl homocysteine lyase that involves the synthesis of auto inducer Al-2, a signaling molecule for quorum sensing in response to population density. Bacteriocin bound to luxS may therefore interfere with signaling communication, and the density of microorganisms will be reduced.

The results of docking from ClusPro are in *S5 Table*. The lowest energy scores among all the clusters for each interaction were selected to compare and illustrate the interactions. Interestingly, Gassericin A and luxS had the lowest energy scores for balanced, electrostatic, hydrophobic, and Van der Waals interactions; these scores were $-1,396.2, -1,400.1, -2,055.9$ and $-142.1$, respectively. The minimum bond length between them in this area was approximately $3.60 \, \text{Å}$ at histidine (HIS-61) of luxS and alanine (ALA-67), as shown in Fig 6. Their binding occurred at the metal binding site. Metal iron is required for luxS metabolism and stability. At this location in luxS (residues 57, 61, and 127), iron ion bound with these interfaces and made the enzyme stable. This *in silico* binding experiment showed that bacteriocin could strongly interfere in the binding of luxS with iron at position 61. In addition, the binding sites of luxS to bacteriocin at amino acid residues 12–157 could prevent binding between luxS and iron. Therefore, a lack of iron bound with luxS causes instability of the enzyme. Although the mode of action of Gassericin A was reported as membrane dissociation of the microorganism, this *in silico* binding result suggested an additional action of Gassericin A via luxS. However, the experiments are required to confirm this preliminary screening.

There were two interesting interactions between bacteriocins (LSEI_2163 and Gassericin A) and gtfB. They returned lower energy scores than LSEI_2386. The respective energy scores of the balanced, electrostatic, hydrophobic, and Van der Waals interactions were close in every mode, and we visualized them with PyMOL to view their binding sites. These two protein-protein interactions occurred at the different sites. Gassericin A’s interaction occurred at a cell wall-binding site, which may not affect the function of this protein. On the other hand, the result showed that LSEI_2163 bound to gtfB’s interface at the catalytic region, as shown in Fig 7. These interaction sites are located at residues (17–23) and (5–15) of LSEI_2163 and residues (493–512) and (446–487) of gtfB. The minimum bond length between them was approximately $1.70 \, \text{Å}$. Bacteriocin could interfere with the production of insoluble glucans at the catalytic region of the enzyme. This analysis showed binding between bacteriocin and the target protein based on a computer model; therefore, further laboratory experiments to confirm the binding, such as yeast two hybrid experiments and investigation of required concentration to control the microorganism, are still required.

The results showed strong bonding between the bacteriocins and the target proteins of *S. mutans*, which supports putative antagonistic activity against *S. mutans* [18, 19]. Therefore, the ab initio prediction of antibacterial activity by computational methods may be an alternative preliminary step before in vitro experimentation. In conclusion, *L. paracasei* SD1 was not found to contain any suspicious genetic material indicating toxicity. Therefore, *L. paracasei* SD1 may be used as a probiotic in dairy products due to its ability to inhibit other bacterial strains.
Fig 6. Protein-protein interaction between luxS and Gassericin A. The interaction between luxS (red) and Gassericin A (blue) was created using ClusPro and visualized by PyMOL. The minimum bond length between them in this area was approximately 3.60 Å at HIS-61 of luxS and ALA-67. The contact sites of luxS and Gassericin A were located at a metal binding site (green) and another site (red).

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Fig 7. Protein–protein interaction between gtfB and LSEI_2163.

The interaction between gtfB (green) and LSEI_2163 (orange) was created by using ClusPro and visualized by PyMOL. The minimum bond length between them was approximately 1.70 Å. The average bond length between them was 1.7–3.6 Å and the lowest energy of the balanced modes from ClusPro was around −1,000.

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Supporting information

S1 Fig. **The bacterial gene and protein toxin identification workflows.** The workflow was constructed with two main processes, a preliminary identification and the local blast and sequence alignment. The preliminary identification was implemented to search related gene and protein by using the BLASTN in the VFDB database and the BLASTX in the DBETH database. Then, the local blast and sequence alignment processes were used to check and confirm the similarity of the sequences of the identification.

(TIF)

S2 Fig. **Circular representation of the chromosome of *Lactobacillus paracasei* SD1 plasmids, plasmid (A) pSD1-1 and (B) pSD1-2.** For both figures A and B, from the inner to outer circles: GC content, AT graph, CDS and Gene. The forward and reverse strand of CDS are yellow color and distinguished by using arrow direction. The ORFs are displayed in orange color.

(TIF)

S1 Table. **List of coding sequences identified in unique regions of the SD1.**

(DOCX)

S2 Table. **The protein toxin sequences in the SD1.** The complete sequences of the Hemolysin, Hemolysin III, and lepB and molecular weight are given, respectively.

(DOCX)

S3 Table. **The report of antibiotic resistance proteins in the SD1.**

(DOCX)

S4 Table. **The bacteriocin sequences in the SD1 genome and plasmid.**

(DOCX)

S5 Table. **The lowest energy from *in silico* binding with the ClusPro between the bacteriocins of the SD1 and luxS and gtfB of *S.mutans*.**

(DOCX)

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Author Contributions

**Data curation:** Komwit Surachat, Panchalika Deachamag.

**Formal analysis:** Komwit Surachat.

**Funding acquisition:** Wilaiwan Chotigeat.

**Investigation:** Komwit Surachat, Panchalika Deachamag.

**Methodology:** Komwit Surachat, Unitsa Sangket, Panchalika Deachamag, Wilaiwan Chotigeat.

**Project administration:** Wilaiwan Chotigeat.

**Resources:** Wilaiwan Chotigeat.
Software: Komwit Surachat, Unitsa Sangket.

Supervision: Unitsa Sangket, Wilaiwan Chotigeat.

Validation: Komwit Surachat, Wilaiwan Chotigeat.

Visualization: Komwit Surachat, Wilaiwan Chotigeat.

Writing – original draft: Komwit Surachat.

Writing – review & editing: Komwit Surachat, Wilaiwan Chotigeat.

References

1. Food, Agriculture Organization of the United Nations WHO. Probiotics in food: health and nutritional properties and guidelines for evaluation. Italy: Rome: Food and Agriculture Organization of the United Nations: World Health Organization, 2006.; 2006. Available from: ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf.

2. Kaushik JK, Kumar A, Duany RK, Mohanty AK, Grover S, Batish VK. Functional and probiotic attributes of an indigenous isolate of Lactobacillus plantarum. PLoS One. 2009;4(12):e8099. https://doi.org/10.1371/journal.pone.0008099 PMID: 19956615

3. Aktas B, De Wolfe TJ, Tandee K, Safdar N, Darien BJ, Steele JL. The Effect of Lactobacillus casei 32G on the Mouse Cecum Microbiota and Innate Immune Response Is Dose and Time Dependent. PLoS One. 2015; 10(12):e0145784. https://doi.org/10.1371/journal.pone.0145784 PMID: 26714177

4. Kwak SH, Cho YM, Noh GM, Om AS. Cancer Preventive Potential of Kimchi Lactic Acid Bacteria (Weissella cibaria, Lactobacillus plantarum). J Cancer Prev. 2014; 19(4):253–8. https://doi.org/10.15430/JCP.2014.19.4.253 PMID: 25574459

5. Davis CD, Milner JA. Gastrointestinal microflora, food components and colon cancer prevention. J Nutr Biochem. 2009; 20(10):743–52. https://doi.org/10.1016/j.jnutbio.2009.06.001 PMID: 19716282

6. Niers LE, Hoekstra MO, Timmerman HM, van Uden NO, de Graaf PM, Smits HH, et al. Selection of probiotic bacteria for prevention of allergic diseases: immunomodulation of neonatal dendritic cells. Clin Exp Immunol. 2007; 149(2):344–52. https://doi.org/10.1111/j.1365-2249.2007.03421.x PMID: 17521319

7. Devine DA, Marsh PD. Prospects for the development of probiotics and prebiotics for oral applications. J Oral Microbiol. 2009; 1. https://doi.org/10.3402/jom.v1i0.1949 PMID: 21523212

8. Terai T, Okumura T, Imai S, Nakao M, Yamaji K, Ito M, et al. Screening of Probiotic Candidates in Human Oral Bacteria for the Prevention of Dental Disease. PLoS One. 2015; 10(6):e0128657. https://doi.org/10.1371/journal.pone.0128657 PMID: 26053410

9. Hynonen U, Palva A. Lactobacillus surface layer proteins: structure, function and applications. Appl Microbiol Biotechnol. 2013; 97(12):5225–43. https://doi.org/10.1007/s00253-013-4962-2 PMID: 23677442

10. Preidis GA, Versalovic J. Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. Gastroenterology. 2009; 136(6):2015–31. https://doi.org/10.1053/j.gastro.2009.01.072 PMID: 19462507

11. Tsai YT, Cheng PC, Pan TM. The immunomodulatory effects of lactic acid bacteria for improving immune functions and benefits. Appl Microbiol Biotechnol. 2012; 96(4):853–62. https://doi.org/10.1007/s00253-012-4407-3 PMID: 23001058

12. Jiang Q, Stamatova I, Kainulainen V, Korpela R, Meurman JH. Interactions between Lactobacillus rhamnosus GG and oral micro-organisms in an in vitro biofilm model. BMC Microbiol. 2016; 16(1):149. https://doi.org/10.1186/s12866-016-0759-7 PMID: 27405227

13. Tapiovaara L, Lehtoranta L, Swanljung E, Makivuokko H, Laakso S, Roivainen M, et al. Lactobacillus rhamnosus GG in the middle ear after randomized, double-blind, placebo-controlled oral administration. Int J Pediatr Otorhinolaryngol. 2014; 78(10):1637–41. https://doi.org/10.1016/j.ijporl.2014.07.011 PMID: 25085073

14. Pan T, Guo HY, Zhang H, Liu AP, Wang XX, Ren FZ. Oral administration of Lactobacillus paracasei alleviates clinical symptoms of colitis induced by dextran sulphate sodium salt in BALB/c mice. Benef Microbes. 2014; 5(3):315–22. https://doi.org/10.3920/BM2013.0041 PMID: 24889689

15. Yi-Knuuttilla H, Snaij J, Kari K, Meurman JH. Colonization of Lactobacillus rhamnosus GG in the oral cavity. Oral Microbiol Immunol. 2006; 21(2):129–31. https://doi.org/10.1111/j.1399-302X.2006.00258.x PMID: 16476023
Analysis of protein toxin and bacteriocins from Lactobacillus paracasei SD1 genome

16. Ciandrini E, Campana R, Casettari L, Perinelli DR, Fagioli L, Manti A, et al. Characterization of biosurfactants produced by Lactobacillus spp. and their activity against oral streptococci biofilm. Appl Microbiol Biotechnol. 2016; 100(15):6767–77. https://doi.org/10.1007/s00253-016-7531-7 PMID: 27102127

17. Wannun P, Piwat S, Teanpaisan R. Purification and characterization of bacteriocin produced by oral Lactobacillus paracasei SD1. Anaerobe. 2014; 27-17–21. https://doi.org/10.1016/j.anaerobe.2014.03.001 PMID: 24636984

18. Teanpaisan R, Piwat S. Lactobacillus paracasei SD1, a novel probiotic, reduces mutants streptococci in human volunteers: a randomized placebo-controlled trial. Clin Oral Investig. 2014; 18(3):857–62. https://doi.org/10.1007/s00784-013-1057-5 PMID: 23892501

19. Riththagol W, Saetang C, Teanpaisan R. Effect of Probiotics Containing Lactobacillus paracasei SD1 on Salivary Mutans Streptococci and Lactobacilli in Orthodontic Cleft Patients: A Double-Blinded, Randomized, Placebo-Controlled Study. Cleft Palate Craniofac J. 2014; 51(3):257–63. https://doi.org/10.1597/12-243 PMID: 23655694

20. Krumsieck J, Arnold R, Rattei T. Gepard: a rapid and sensitive tool for creating dotplots on genome scale. Bioinformatics. 2007; 23(8):1026–8. https://doi.org/10.1093/bioinformatics/btm039 PMID: 17309866

21. Hunt M, Silva ND, Otto TD, Parkhill J, Keane JA, Harris SR. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol. 2015; 16:294. https://doi.org/10.1186/s13059-015-0849-0 PMID: 26714481

22. Delcher AL, Bratke KA, Powers EC, Salzberg SL. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics. 2002; 23(6):673–9. https://doi.org/10.1093/bioinformatics/btq1226 PMID: 12737039

23. Lukashin AV, Borodovsky M. GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res. 1998; 26(4):1107–15. https://doi.org/10.1093/nar/26.4.1107 PMID: 9461475

24. Besemer J, Borodovsky M. Heuristic approach to deriving models for gene finding. Nucleic Acids Res. 1999; 27(19):3911–20. https://doi.org/10.1093/nar/27.19.3911 PMID: 10481031

25. Besemer J, Lomsadze A, Borodovsky M. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. 2001; 29(12):2607–18. https://doi.org/10.1093/nar/29.12.2607 PMID: 11410670

26. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics. 2008; 9:75. https://doi.org/10.1186/1471-2164-9-75 PMID: 18261238

27. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2014; 42(Database issue):D206–14. https://doi.org/10.1093/nar/gkt1226 PMID: 24293654

28. Lowe TM, Chan PP. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 2016; 44(W1):W54–7. https://doi.org/10.1093/nar/gkw413 PMID: 27174935

29. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 1997; 25(5):955–64. https://doi.org/10.1093/nar/25.5.955 PMID: 9023104

30. Schattner P, Brooks AN, Lowe TM. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res. 2005; 33(Web Server issue):W686–9. https://doi.org/10.1093/nar/gki366 PMID: 15980563

31. Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 2007; 35(9):3100–8. https://doi.org/10.1093/nar/gkm160 PMID: 17452365

32. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. PHAST: a fast phage search tool. Nucleic Acids Res. 2011; 39(Web Server issue):W347–52. https://doi.org/10.1093/nar/gkr485 PMID: 21672955

33. Grissa I, Vergnaud G, Pourcel C. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res. 2007; 35(Web Server issue):W52–7. https://doi.org/10.1093/nar/gkm360 PMID: 17537822

34. Grant JR, Stothard P. The CGView Server: a comparative genomics tool for circular genomes. Nucleic Acids Res. 2008; 36(Web Server issue):W181–4. https://doi.org/10.1093/nar/gkn179 PMID: 18411202

35. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004; 32(5):1792–7. https://doi.org/10.1093/nar/gkh340 PMID: 15034147

36. Kearse M; 2012. Available from: http://www.geneious.com.
38. Chen L, Xiong Z, Sun L, Yang J, Jin Q. VFDB 2012 update: toward the genetic diversity and molecular evolution of bacterial virulence factors. Nucleic Acids Res. 2012; 40(Database issue):D641–5. https://doi.org/10.1093/nar/gkr989 PMID: 22067448

39. Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, et al. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res. 2005; 33(Database issue):D325–8. https://doi.org/10.1093/nar/gki008 PMID: 15608208

40. Chen L, Zheng D, Liu B, Yang J, Jin Q. VFDB 2016: hierarchical and refined dataset for big data analysis–10 years on. Nucleic Acids Res. 2016; 44(D1):D694–7. https://doi.org/10.1093/nar/gkv1239 PMID: 26578559

41. Yang J, Chen L, Sun L, Yu J, Jin Q. VFDB 2008 release: an enhanced web-based resource for comparative pathogenomics. Nucleic Acids Res. 2008; 36(Database issue):D539–42. https://doi.org/10.1093/nar/gkm951 PMID: 17984080

42. Chakraborty A, Ghosh S, Chowdhary G, Maulik U, Chakrabarti S. DBETH: a Database of Bacterial Exotoxins for Human. Nucleic Acids Res. 2012; 40(Database issue):D615–20. https://doi.org/10.1093/nar/gkr942 PMID: 22102573

43. Bi D, Xu Z, Harrison EM, Tai C, Wei Y, He X, et al. ICEberg: a web-based resource for integrative and conjugal elements found in Bacteria. Nucleic Acids Res. 2012; 40(Database issue):D621–6. https://doi.org/10.1093/nar/gkr846 PMID: 22009673

44. Gibson MK, Forsberg KJ, Dantas G. Improved annotation of antibiotic resistance determinants reveals microbial resistomes cluster by ecology. ISME J. 2015; 9(1):207–16. https://doi.org/10.1038/ismej.2014.106 PMID: 25003965

45. de Jong A, van Hijum SA, Bijlsma JJ, Kok J, Kuipers OP. BAGEL: a web-based bacteriocin genome mining tool. Nucleic Acids Res. 2006; 34(Web Server issue):W273–9. https://doi.org/10.1093/nar/gkl237 PMID: 16845009

46. Hammami R, Zouhir A, Ben Hamida J, Fliss I. BACTIBASE: a new web-accessible database for bacteriocin characterization. BMC Microbiol. 2007; 7:89. https://doi.org/10.1186/1471-2180-7-89 PMID: 17941971

47. Hammami R, Zouhir A, Le Lay C, Ben Hamida J, Fliss I. BACTIBASE second release: a database and tool platform for bacteriocin characterization. BMC Microbiol. 2010; 10:22. https://doi.org/10.1186/1471-2180-10-22 PMID: 20105292

48. Kallberg M, Margaryan G, Wang S, Ma J, Xu J. RaptorX server: a resource for template-based protein structure modeling. Methods Mol Biol. 2014; 1137:17–27. https://doi.org/10.1007/978-1-4939-0366-5_2 PMID: 24573471

49. Kallberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, et al. Template-based protein structure modeling using the RaptorX web server. Nat Protoc. 2012; 7(8):1511–22. https://doi.org/10.1038/nprot.2012.085 PMID: 22814390

50. Peng J, Xu J. RaptorX: exploiting structure information for protein alignment by statistical inference. Proteins. 2011; 79 Suppl 1:617–71. https://doi.org/10.1002/prot.23175 PMID: 21987485

51. Wang S, Li W, Liu S, Xu J. RaptorX-Property: a web server for protein structure property prediction. Nucleic Acids Res. 2016; 44(W1):W430–5. https://doi.org/10.1093/nar/gkw306 PMID: 27112573

52. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. Nat Protoc. 2010; 5(4):725–38. https://doi.org/10.1038/nprot.2010.5 PMID: 20360767

53. Yang J, Zhang Y. I-TASSER server: new development for protein structure and function predictions. Nucleic Acids Res. 2015; 43(W1):W174–81. https://doi.org/10.1093/nar/gkv342 PMID: 25883148

54. Yang J, Zhang Y. Protein Structure and Function Prediction Using I-TASSER. Curr Protoc Bioinformatics. 2015; 52:5.8.1–15. https://doi.org/10.1002/0471250953.bi0508s52

55. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics. 2008; 9:40. https://doi.org/10.1186/1471-2105-9-40 PMID: 18215316

56. Zhang Y. I-TASSER: fully automated protein structure prediction in CASP8. Proteins. 2009; 77 Suppl 9:100–13. https://doi.org/10.1002/prot.22586 PMID: 19768667

57. Xu D, Zhang Y. Ab initio protein structure assembly using continuous structure fragments and optimized knowledge-based force field. Proteins. 2012; 80(7):1715–35. https://doi.org/10.1002/prot.24065 PMID: 22411565

58. Lovell SC, Davis IW, Arendall W B, de Bakker PI, Word JM, Prisant MG, et al. Structure validation by Calpha geometry: phi, psi and Cbeta deviation. Proteins. 2003; 50(3):437–50. https://doi.org/10.1002/prot.10286 PMID: 12557186

59. Laskowski RA, Rullmann JA, MacArthur MW, Kaptein R, Thornton JM. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. J Biomol NMR. 1996; 8(4):477–86. https://doi.org/10.1007/BF0028148 PMID: 9008363
60. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics. 2006; 22(2):195–201. https://doi.org/10.1093/bioinformatics/btl770 PMID: 16301204

61. Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. Nucleic Acids Res. 2014; 42 (Web Server issue):W252–8. https://doi.org/10.1093/nar/gku340 PMID: 24782522

62. Kopp J, Schwede T. The SWISS-MODEL Repository of annotated three-dimensional protein structure homology models. Nucleic Acids Res. 2004; 32(Database issue):D230–4. https://doi.org/10.1093/nar/gkh008 PMID: 14681401

63. Kopp J, Schwede T. The SWISS-MODEL Repository: new features and functionalities. Nucleic Acids Res. 2006; 34(Database issue):D315–8. https://doi.org/10.1093/nar/gkj056 PMID: 16381875

64. Schwede T, Kopp J, Greue N, Peiltsch MC. SWISS-MODEL: An automated protein homology-modeling server. Nucleic Acids Res. 2003; 31(13):3381–5. https://doi.org/10.1093/nar/gkg520 PMID: 12824332

65. Kozakov D, Brenke R, Comeau SR, Vajda S. PIPER: an FFT-based protein docking program with pairwise potentials. Proteins. 2006; 65(2):392–406. https://doi.org/10.1002/prot.21117 PMID: 16933295

66. Comeau SR, Gatchell DW, Vajda S, Camacho CJ. ClusPro: a fully automated algorithm for protein-protein docking. Nucleic Acids Res. 2004; 32(Web Server issue):W96–9. https://doi.org/10.1093/nar/gkh354 PMID: 15215358

67. Comeau SR, Gatchell DW, Vajda S, Camacho CJ. ClusPro: an automated docking and discrimination method for the prediction of protein complexes. Bioinformatics. 2004; 20(1):45–50. https://doi.org/10.1093/bioinformatics/btg371 PMID: 14693807

68. Comeau SR, Kozakov D, Brenke R, Shen Y, Beglov D, Vajda S. ClusPro: performance in CAPRI rounds 6–11 and the new server. Proteins. 2007; 69(4):781–5. https://doi.org/10.1002/prot.21795 PMID: 17876812

69. Schrodinger L. The PyMOL Molecular Graphics System, Version 1.8. 2015.

70. Dhillon BK, Laird MR, Shay JA, Winsor GL, Lo R, Nizam F, et al. IslandViewer 3: more flexible, interactive genomic island discovery, visualization and analysis. Nucleic Acids Res. 2015; 43(W1):W104–8. https://doi.org/10.1093/nar/gkv401 PMID: 25916842

71. Kawai Y, Kemperman R, Kok J, Saito T. The circular bacteriocins gassericin A and circularin A. Curr Dairy Sci. 2007; 95(10):5495–509. https://doi.org/10.3168/jds.2011-4756 PMID: 22863096

72. Hsu S. Effect of ferric iron on siderophore production and pyrene degradation by Pseudomonas fluorescens 29L. Curr Microbiol. 2008; 57(4):331–4. https://doi.org/10.1007/s00284-008-9198-5 PMID: 18626691

73. Vesterlund S, Vankercikhoven V, Saxelin M, Goossens H, Salminen S, Ouwehand AC. Safety assessment of Lactobacillus strains: presence of putative risk factors in faecal, blood and probiotic isolates. Int J Food Microbiol. 2007; 116(3):325–31. https://doi.org/10.1016/j.ijfoodmicro.2007.02.002 PMID: 17391794

74. Zhang ZY, Liu C, Zhu YZ, Wei YX, Tian F, Zhao GP, et al. Safety assessment of Lactobacillus plantarum JDM1 based on the complete genome. Int J Food Microbiol. 2012; 153(1–2):166–70. https://doi.org/10.1016/j.ijfoodmicro.2011.11.003 PMID: 22133564

75. Senan S, Prajapati JB, Joshi CG. Feasibility of Genome-Wide Screening for Biosafety Assessment of Probiotics: A Case Study of Lactobacillus helveticus MTCC 5463. Probiotics Antimicrob Proteins. 2015; 7(4):249–58. https://doi.org/10.1007/s12602-015-9199-1 PMID: 26223907

76. Rodriguez da Cunha L, Fortes Ferreira CL, Durmaz E, Goh YJ, Sanozky-Dawes R, Klaenhammer T. Characterization of Lactobacillus gasseri isolates from a breast-fed infant. Gut Microbes. 2012; 3(1):15–24. https://doi.org/10.4161/gmic.19489 PMID: 22655546

77. Ouwehand AC, Saxelin M, Salminen S. Phenotypic differences between commercial Lactobacillus rhamnosus GG and L. rhamnosus strains recovered from blood. Clin Infect Dis. 2004; 39(12):1858–60. https://doi.org/10.1086/425741 PMID: 15578412
81. Cotter PD, Hill C, Ross RP. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol. 2005; 3(10):777–88. https://doi.org/10.1038/nrmicro1273 PMID: 16205711

82. Kuo YC, Liu CF, Lin JF, Li AC, Lo TC, Lin TH. Characterization of putative class II bacteriocins identified from a non-bacteriocin-producing strain Lactobacillus casei ATCC 334. Appl Microbiol Biotechnol. 2013; 97(1):237–46. https://doi.org/10.1007/s00253-012-4149-2 PMID: 22688903

83. Hu CB, Malaphan W, Zendo T, Nakayama J, Sonomoto K. Enterocin X, a novel two-peptide bacteriocin from Enterococcus faecium KU-BS, has an antibacterial spectrum entirely different from those of its component peptides. Appl Environ Microbiol. 2010; 76(13):4542–5. https://doi.org/10.1128/AEM.02264-09 PMID: 20418437

84. Borrero J, Jiménez JJ, Gutierrez L, Herranz C, Cintas LM, Hernandez PE. Protein expression vector and secretion signal peptide optimization to drive the production, secretion, and functional expression of the bacteriocin enterocin A in lactic acid bacteria. J Biotechnol. 2011; 156(1):76–86. https://doi.org/10.1016/j.jbiotec.2011.07.038 PMID: 21839785

85. Pandey N, Malik RK, Kaushik JK, Singroha G. Gassericin A: a circular bacteriocin produced by Lactic acid bacteria Lactobacillus gasseri. World Journal of Microbiology and Biotechnology. 2013; 29(11):1977–1987. https://doi.org/10.1007/s11274-013-1368-3 PMID: 23712477

86. Nakamura K, Arakawa K, Kawai Y, Yasuta N, Chuo T, Watanabe M, et al. Food preservative potential of gassericin A-containing concentrate prepared from cheese whey culture supernatant of Lactobacillus gasseri LA39. Animal Science Journal. 2013; 84(2):144–149. https://doi.org/10.1111/j.1740-0929.2012.01048.x PMID: 23384356

87. Cai H, Thompson R, Budinch MF, Broadbent JR, Steele JL. Genome sequence and comparative genome analysis of Lactobacillus casei: insights into their niche-associated evolution. Genome Biol Evol. 2009; 1:239–57. https://doi.org/10.1093/gbe/evp019 PMID: 20333194

88. Ito Y, Kawai Y, Arakawa K, Homme Y, Sasaki T, Saito T. Conjugative plasmid from Lactobacillus gasseri LA39 that carries genes for production of and immunity to the circular bacteriocin gassericin A. Appl Environ Microbiol. 2009; 75(19):6340–51. https://doi.org/10.1128/AEM.00195-09 PMID: 19666732

89. Kawai Y, Saito T, Suzuki M, Itoh T. Sequence analysis by cloning of the structural gene of gassericin A, a hydrophobic bacteriocin produced by Lactobacillus gasseri LA39. Biosci Biotechnol Biochem. 1998; 62(5):887–92. https://doi.org/10.1271/bbb.62.887 PMID: 9648219

90. Pandey N, Malik RK, Kaushik JK, Singroha G. Gassericin A: a circular bacteriocin produced by lactic acid bacteria Lactobacillus gasseri. World J Microbiol Biotechnol. 2013; 29(11):1977–87. https://doi.org/10.1007/s11274-013-1368-3 PMID: 23712477

91. Gabrielsen C, Brede DA, Nis IF, Diep DB. Circular bacteriocins: biosynthesis and mode of action. Appl Environ Microbiol. 2014; 80(22):6854–62. https://doi.org/10.1128/AEM.02284-14 PMID: 25172850

92. Kawai Y, Ishii Y, Arakawa K, Uemura K, Saitoh B, Nishimura J, et al. Structural and functional differences in two cyclic bacteriocins with the same sequences produced by lactobacilli. Appl Environ Microbiol. 2004; 70(5):2906–11. https://doi.org/10.1128/AEM.70.5.2906-2911.2004 PMID: 15128550

93. Ahmed A, Dachang W, Lei Z, Jianjun L, Jianshen O, Yi X. Effect of Lactobacillus species on Streptococcus mutans biofilm formation. Pak J Pharm Sci. 2014; 27(Spec no):1523–8. PMID: 25176247

94. Diep DB, Skauen M, Salehian Z, Holo H, Nis IF. Common mechanisms of target cell recognition and immunity for class II bacteriocins. Proc Natl Acad Sci U S A. 2007; 104(7):2384–9. https://doi.org/10.1073/pnas.0608775104 PMID: 17284603