International Scholars Journals

International Journal of Microbiology Research and Reviews ISSN 2329-9800, Vol. 13 (1), pp. 001-008, June, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Impairment of Lactococcus lactis Growth, Sugar Metabolism, and Acid Resistance Due to Disruption of the Signal Recognition Particle Pathway

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Accepted 16 June, 2025

The well-known dairy product workhorse Lactococcus lactis has significant industrial characteristics that are closely linked to a variety of cytoplasmic membrane proteins. However, no research has been done on the functions of the signal recognition particle (SRP) pathway, which targets membrane proteins, in Lactobacillus lactis. This study found the putative genes ffh and ftsY that encode components of the SRP pathway in the genome of Lactobacillus lactis NZ9000. Experiments revealed that while neither the ffh nor the ftsY sequence mutation was fatal, it did lengthen the lag phase of the resulting mutants Δffh and ΔftsY by two hours, reduce their biomass to 85.7% of the wild type in static conditions, and prevent the mutants from growing more efficiently in aerobic respiration. In addition, the mutants exhibited markedly reduced rates of lactate generation and glucose consumption. The effect of the SPR components on acid resistance was then identified, revealing that during acid challenge at pH 3.0, the ffh and ftsY were transcriptionally upregulated by 3.02 ± 1.21 and 8.66 ± 1.01 times in the wild type, respectively, and that the Δffh and ΔftsY cell survival dropped by 10 and 100 times in comparison to the wild type. Proteomics analysis was conducted to investigate the potential mechanism of the SRP pathway involved in the aforementioned physiological traits. The results showed that disruption of the Ffh or FtsY resulted in an increase in DnaK, GroEL, and heat shock protein GrpE, but a decrease in ribosomal proteins. This suggests that the SRP pathway was closely associated with protein synthesis and folding in Lactobacillus lactis. Reduced levels of respiratory complexes NADH dehydrogenase, fructose-bisphosphate aldolase, and glutamate decarboxylase were also seen in the Δffh and ΔftsY, which is in line with the phenomena of acid resistance and poor sugar metabolism. Our findings showed that the dispensable SRP pathway may support L. lactis's acid resistance and metabolism balance.

Key words: Lactococcus lactis, SRP pathway, Ffh, FtsY, Acid resistance.

INTRODUCTION

A common lactic acid bacterium (LAB) utilized as a starter strain in the manufacturing of cheese, buttermilk, and sour cream is Lactococcus lactis, which helps to quickly generate lactate and alter its flavor and texture (Li et al., 2020; Coelho et al., 2022; Kongo, 2013). From starter preparation to dairy fermentation, L. lactis is predicted to function like a workhorse due to its enormous economic importance (A´lvarez-Martín et al., 2008; Sulieman, 2022). The key physiological characteristics, including the use of sugar, aerobic respiration, and tolerance to various industrial stressors, have thus been the focus of significant attention (Zhu et al., 2009). All things considered, many cytoplasmic membrane proteins are intimately associated with these physiological activities (Zhang et al., 2012; Clausen et al., 2002). For instance, a minimal electron

transport chain for L. lactis respiration is made up of the integral membrane proteins NADH-dehydrogenase, bd-type cytochrome, and menaquinone-pool (Rezaïki et al., 2004; Gaudu et al., 2002). The cytoplasmic membrane contains a variety of transport mechanisms that are essential for both acid tolerance and sugar absorption (Gaudu et al., 2002). Therefore, the maintenance of these crucial industrial characteristics depends on the effective integration of proteins into the cytoplasmic membrane.

Membrane and secretory protein localization is primarily controlled by the signal recognition particle (SRP) pathway, which is conversed throughout all three kingdoms of life (Kellogg et al., 2021). The N-terminal hydrophobic signal sequence of a developing ribosome-nascent chain (RNC) is recognized and bound by the bacterial SRP, which is made up of the SRP protein Ffh and 4.5S RNA. The SRP-RNC complex then binds with the SRP receptor FtsY, releasing the nascent chain to the SecYEG translocon and facilitating either target integration into the cytoplasmic membrane concurrent with translation or protein translocation across the membrane (Wild et al., 2004). The misfolding of polypeptides in the crowded cytoplasm may be avoided by appropriately translocating rapid translational polypeptide chains (Hegde and Keenan, 2022; Zhang and Shan, 2014). The SRP route is frequently seen as crucial (Wild et al., 2004). Since insertase YidC2 may partially compensate for the SRP pathway's activity, Streptococcus mutans observations revealed that disruption of the process typically did not result in a complete block of membrane protein insertion (Hasona et al., 2005; Mishra et al., 2019). Depletion of the Ffh led to decreased protein synthesis and overall cell fitness, according to research done on Escherichia coli (Wickstrom et al., 2011). Additionally, extracellular protein accumulation was impacted by Bacillus subtilis Ffh and FtsY reduction (Zanen et al., 2006). Little is known about the SRP route in other bacteria, and the necessity of the SRP pathway is still up for debate.

L. lactis undergoes genome destruction as pseudogenes appear during long-term adaptation to milk, and it also acquires particular genes involved in lactose fermentation and casein breakdown (Zhu et al., 2009; Cavanagh et al., 2015; Kleerebezem et al., 2020). Although their functions have not been investigated, genome annotation revealed that the conserved SRP pathway components Ffh and FtsY are still present in the L. lactis genome. The purpose of this study was to assess the SRP pathway's essentiality in L. lactis. It was also shown that L. lactis Ffh and FtsY contributed to acid resistance, sugar metabolism, and cell proliferation. Additionally, the impact of Ffh or FtsY disruption on the proteome of Lactobacillus lactis was examined. According to our findings, Ffh and FtsY are not necessary for L. lactis cell survival, but they are strongly linked to acid resistance and homeostasis in metabolism.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Table 1 provides a summary of the bacterial strains and plasmids used in this study. The plasmid-building E. Coli XL1-Blue was cultured aerobically at 37 °C in Luria Bertani broth. L. lactis NZ9000 and its derivatives were regularly grown at 30 °C under static conditions using M17 broth (Oxoid, Basingstoke, United Kingdom) that contained 0.5% (wt/vol) glucose (GM17). L. lactis was grown in GM17 broth with 2 mg/L of heme at 30 °C on a rotary shaker at 200 rpm in order to initiate aerobic respiration. When required, erythromycin (Sangon, China) was used at 10 μg/mL for Lactobacillus lactis or 250 μg/mL for Escherichia coli, and chloramphenicol (Sangon, China) at 5 μg/mL for Lactobacillus lactis or 10 μg/mL for E. coli. The final concentration of nisin (Sigma, USA) was 10 ng/mL. When necessary, HCl was added to GM17 broth to bring its natural pH of 7.0 down to 3.0.

2.2. Sequence analysis of the L. lactis ffh and ftsY

The S. mutans and E. coli ffh and ftsY genes were used as queries to search the L. lactis NZ9000 genome for the putative ffh and ftsY genes. ESPript 3.0 and Clustal W were used for multiple-sequence alignments. According to the prior report, Operon prediction was carried out (Taboada et al., 2018). The websites of Softberry (http://www.softberry.com/) and ARNold (http://rssf.i2bc.paris-sa clay.fr/toolbox/arnold/index.php) were used for promoter and terminator prediction, respectively. Alphafold2 was used to simulate the protein structures of the L. lactis FtsY and Ffh. The MEGA program was used to construct the phylogenetic tree (Kumar et al., 2016).

2.3. Plasmid construction and gene mutation

Table 2 contains a list of the primers and oligonucleotides utilized in this study. Plasmid Mini Kit (Omega) and TIANamp Bacteria DNA Kit (TIANGEN, China) were used for plasmid extraction and bacterial genomic DNA extraction, respectively. T4 poly-nucleotide kinase and T4 DNA ligase, restriction enzymes, were employed in accordance with New England Biolabs' standard operating procedures (NEB, USA). We bought Taq polymerase for screening and high-fidelity DNA polymerase for cloning from Takara (Japan) and NEB, respectively. The company RuiBiotech Biological Biotechnology Ltd. (China) carried out the DNA synthesis and sequencing.

As our earlier strategy, we used CRISPR-Cas9 assisted ssDNA recombination to perform mutations in the ffh and ftsY of L. lactis NZ9000 (Guo et al., 2019). The oligonucleotides Ffh-DSBF/Ffh-DSBR and FtsY-DSBF/FtsY-DSBR were used to create the target plasmids pTHCas9ffh and pTHCas9ftsY, respectively. Target plasmid pTHCasffh and oligonucleotides ssDNA-ffh were used to introduce two consecutive stop codons in the ffh (llnz_04520) in the

genome of L. lactis NZ9000, resulting in the mutant strain L. lactis Δffh. Similarly, oligonucleotides ssDNA-ftsY and the target plasmid pTHCas9ftsY were used to remove the 100 bp in-frame DNA fragment of the ftsY (llnz_09010) in the genome of L. lactis NZ9000, creating the mutant strain L. lactis ΔftsY. By using primers Ffhcexu-F/Ffhcexu-R for PCR amplification and FtsYcexu-F/FtsYcexu-R for sequencing, the mutations in L. lactis Δffh and L. lactis ΔftsY were verified.

2.4. Determination of growth, glucose consumption and lactate production

GM17 broth was used to cultivate L. lactis NZ9000, the Δffh, and the ΔftsY in either static or aerobic respiratory conditions. OD600nm was used to monitor cell proliferation. Using glucose and lactate enzyme membranes, the biosensor analyzer (Shandong Academy of Sciences, Jinan) measured the amounts of glucose and lactate in the culture supernatants.

2.5. Transcriptional levels of the ffh and ftsY under acid stress

The cell pellets were collected by centrifugation at 6000g for 5 min and washed twice with PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, pH 7.4). The cell pellets were resuspended in GM17 broth of natural pH and pH = 3.0, and cultivated for 2 h. Then, the cells were collected and total RNA was extracted by using a RNAprep Pure Cell/Bacteria Kit (Tiangen, Beijing, China) following the protocols of the manufacturer. Subsequently, the RNA was reversely transcribed to according to the instructions of the manufacturer. Primer pairs ffhdl-F/ ffhdl-R and ftsYdl-F/ftsYdl-R were used to detect the transcriptional levels of the ffh and ftsY, respectively. The 16S rRNA gene used as an internal standard was amplified with the primer pair 16S-F/16S-R.

2.6. Determination of acid resistance

In GM17 broth, L. lactis NZ9000, Δffh, and ΔftsY were grown until the OD600nm reached 1.0. After being collected by centrifugation at 6000g for five minutes, the cell pellets were twice washed with PBS buffer and then resuspended in GM17 broth with a pH of 3.0. Viable cells were counted by colony forming units (CFU) every 30 minutes while the cells were cultured at 30 °C.

2.7. Protein extraction and SDS-PAGE

A 5 mL culture was centrifuged at 8000g for 10 minutes in order to extract the L. lactis cells from the supernatant. (1) 10% trichloroacetic acid was added to the supernatant in order to concentrate the extracellular proteins. Following two hours in an ice bath, the extracellular proteins were separated by centrifugation at 12,000 g for 20 minutes. They were then cleaned with ice-cold acetone and reconstituted in 100 μL of PBS buffer. (2) To get an OD600nm near 1.0, the cell pellets were resuspended in PBS buffer after two rounds of washing. Glass beads (Sigma-Aldrich, St. Louis, MO) were added to an aliquot of cell suspension, and the cells were crushed by shaking it in a bullet blender (Precellys 24, Bertin, France). The intracellular proteins were extracted from the clear supernatant by centrifuging the cell lysate for 10 minutes at 12,000 g. SDS-PAGE was performed on aliquots of the intracellular and extracellular proteins. Following electrophoresis, Coomassie brilliant blue was used to dye the gel.

2.8. LC-MS/MS

Following SDS-PAGE separation of the intracellular proteins, the gel segment corresponding to each sample lane was removed, un-stained, and cleaned. Iodoacetamide was used to alkylate the proteins in the gel, and trypsin was used to breakdown them. After sonicating the peptides in the gel into a 20 mM NH4HCO3 solution, ZipTip C18 columns were used to desalt the mixture. An LTQ-Orbitrap Velos Pro ETD mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Prominence nano LC system (Shimadzu, Kyoto, Japan) were used to perform LC-MS/MS analysis on the desalted peptides after they had been dissolved with 0.1% (v/v) trifluoroacetic acid.

As previously mentioned, the data searches were carried out using Proteome Discover software, version 1.4 (Thermo Fisher Scientific, USA). The NCBI database was used to download the L. lactis NZ9000 reference database. Peptides exhibiting at least six amino acid residues are the only ones.

The results reflected a 95% certainty level. For protein identification, at least two peptides have to be taken into account. A prior work used peptide-spectrum matches (PSMs) to determine the relative abundance of proteins (Liu et al., 2018).

2.9. Statistical analysis

The mean ± standard deviation was used to report the experimental data. Unpaired 2-tailed Student's t-tests were used to determine whether there was a statistically significant difference between the treatment and control conditions. P less than 0.05 was regarded as statistically significant.

3. Results

3.1. Sequence analysis of homologues of Lactobacillus lactis containing the identified SRP components

The putative ffh and ftsY of single copy were found in the genome of L. lactis NZ9000 using the S. mutans and E. coli ffh and ftsY genes as queries. Genetic structure predicted that they were in distinct operons. A leucine-rich repeat domain-containing protein and ribonuclease J, a potential contributor for the maturation of 4.5S RNA, were anticipated to be the deduced amino acid sequences of putative genes upstream of the ffh gene (Vandanashree et al., 2023). Upstream of the ftsY were the putative genes encoding a hydrolase and hypothetical protein of the cof-type HAD-IIB family, whereas downstream were the genes encoding ribose-phosphate diphosphokinase (Fig. 1A).

There were 518 and 459 amino acids in L. lactis Ffh and FtsY, respectively. According to sequence alignment, the L. lactis Ffh and FtsY shared 67.37% and 46.11% of their identities with S. mutans UA159, 48.53% and 37.03% with E. coli MG1655, and 32.82% and 27.04% with Saccharomyces cerevisiae S288C (Figs. S1 and S2). Additionally, according to phylogenetic tree analysis, the Ffh and FtsY of Lactobacillus lactis had the closest kinship with those of S. thermophilus and S. mutans (Figs. 1B and S3A and B). A GTPase domain and the ribonuclease E domain were predicted by Alphafold2's protein structural model of the L. lactis FtsY (Fig. S4A), as well as the N-terminal helical bundle domain and GTPase domain of the Ffh (Fig. S4B). According to these findings, Ffh and FtsY may be components of the SRP pathway in Lactobacillus lactis.

3.2. Disruption of Ffh or FtsY is not lethal in L. lactis

In order to investigate whether Ffh or FtsY is required for L. lactis growth, two consecutive stop codons were added to the ffh, and using CRISPR-Cas9 assisted ssDNA recombineering technology, 100 bp in-frame DNA deletion was carried out in the ftsY (Fig. 2A and B). This resulted in mutant strains L. lactis Δffh and L. lactis ΔftsY. However, the comparable strategy failed to produce double mutations in both the ffh and the ftsY (data not shown).

Under static circumstances, the development of the resulting mutant strains L. lactis Δffh and L. lactis ΔftsY was assessed in GM17 broth. In contrast to the wild type (WT), both mutants were able to grow, as seen in Fig. 2C. However, when the Ffh or FtsY was disrupted, the mutants' lag phase lasted longer—from 2 to 4 hours—and their stationary phase lasted longer—from 6 to 8 hours. Additionally, the L. lactis Δffh and ΔftsY final cell densities were around 85.7% of the WT. These findings showed that while interference with the Ffh or FtsY does not cause death, it does impede growth performance.

When exogenous heme is present in aerobic conditions, L. lactis conducts respiration and provides the minimal electron transfer chain (Lechardeur et al., 2011). When respiration was turned on, the growth curves of the WT and its mutations were shown in Fig. 2D. The biomass rose by 70.51% and the log phase of the WT culture under respiration conditions lasted for 12 hours in comparison to the static culture. Static and respiration cultures did not show any significant growth differences for either Δffh or ΔftsY, indicating that disruption of L. lactis Ffh or FtsY had an adverse effect on growth in aerobic respiration conditions.

3.3. Following disruption of the Ffh or FtsY, glucose intake and lactate generation

The WT and mutants were compared in terms of glucose consumption and lactate generation in order to examine the impact of the SRP pathway on L. lactis's sugar metabolism. After 6 hours of cultivation, the WT ran out of glucose under static conditions, and 1.50 g/L of lactate was produced. In contrast, the rates of glucose intake

Lactate concentrations in the Δffh and ΔftsY were 1.49 g/L and 1.50 g/L, respectively, and lactate production was reduced in the Δffh and worse in the ΔftsY (Fig. 3A and B). The cultures of WT, Δffh, and ΔftsY had final pH values of 4.67, 4.68, and 4.67, respectively. The ΔftsY experienced more severe effects and the mutants' glucose consumption exhibited slower rates than the WT's under aerobic respiration settings (Fig. 3C). With final pH values of 5.18, 4.70, and 4.67, the WT's lactate content peaked at 6 hours and gradually declined, while the mutant strains did not exhibit lactate reduction (Fig. 3D). This finding suggested that L. lactis's ability to breathe aerobically was hampered by the lack of Ffh or FtsY.

3.4. The SRP pathway's effects on Lactobacillus lactis's acid resistance

The transcriptional levels of ffh and ftsY were examined in order to investigate the effects of the SRP pathway on acid stress in L. lactis. The ffh transcription was up-regulated by 2.21-fold and 3.02-fold at the acid stress for 0.5 and 2 hours, respectively, while the ftsY transcriptional levels increased even more markedly (2.81-fold and 8.66-fold) when the WT was exposed to acid challenge, as indicated in Table 3. Additionally, following acid stress for 0.5 and 2 hours, the ftsY transcription of the strain Δffh grew by 11.19 and 17.62 times, and the ffh transcription of the strain ΔftsY increased by 15.34 and 15.27 times. These findings showed that the SRP pathway components were expressed in response to acid stress and that the ftsY gene was more vulnerable to acid challenge than the ffh gene. The acid stress was used to assess cell survival. As shown in Fig. 4, viable cell numbers of the WT decreased by 102- and 105-fold after 0.5 h and 2 h acid stress, while the numbers of the Δffh decreased by 103-and 106-fold, and the ΔftsY decreased by 103-and 107-fold, indicating that disruption of the key SRP a Relative gene expression was calculated using 16S rRNA as the internal standard gene at the normal condition of L. lactis NZ9000 as the reference.

b Significant difference ($P < 0.05$) in relative gene expression in different strains for the same acid stress time.

c Significant difference ($P < 0.05$) in relative gene expression in the same strain for different acid stress time.

3.5. Proteomics analysis of the Δffh and ΔftsY

The extracellular and intracellular proteins of the WT and the mutants Δffh and ΔftsY were separated and put through SDS-PAGE analysis in order to determine the impact of the SRP pathway disruption on protein synthesis in L. lactis. Protein bands ranging from 45 to 66 kDa were seen in all three samples, as illustrated in Fig. 5, with no discernible difference between the WT and mutant extracellular portions. In contrast, the WT and mutants have distinct intracellular fraction compositions. It is evident that the Δffh and ΔftsY both had additional protein bands of roughly 66 kDa, and the Δffh also had an additional band of 25–30 kDa.

PSM was used to assess changes in the relative abundance of proteins after LC-MS/MS analysis revealed that the intracellular fractions of the WT, Δffh, and ΔftsY included 511, 633, and 725 proteins, respectively (Tables S1, S2, and S3). As Table 4 illustrates, ribosomal proteins were less accumulated in the Δffh and ΔftsY, suggesting that protein synthesis was slower and misfolding was more severe due to disruption of the Ffh and FtsY. In contrast, the DnaK, GroEL, heat shock protein GrpE involved in chaperone, and ATP-dependent RNA helicase involved in ribosome assembly were more accumulated in the Δffh and FtsY. Additionally, there was an increase in DNA polymerase I and the DNA repair protein RecN in the Δffh and ΔftsY, indicating higher DNA damage in these cells. The reduced sugar metabolism and aerobic respiration abilities of the Δffh and ΔftsY were consistent with the markedly reduced accumulation levels of fructose-bisphosphate aldolase and 1-phosphofructokinase involved in glycolysis, NADH de-hydrogenase, and F0F1 ATP synthase subunits involved in oxidative phosphorylation. Adenylosuccinate lyase and glutamate decarboxylase, two enzymes involved in glutamate and arginine metabolism, were similarly reduced in the Δffh and ΔftsY, which would result in weaker acid tolerance. Several PTS components for membrane transporter proteins were reduced in the Δffh and ΔftsY, likely impacting the carbon source intake mechanism. However, the Δffh and ΔftsY revealed a large increase in the accumulation level of the cellobiose-specific PTS system IIC component, which may serve as a supplement for the defective transporters (Castro et al., 2009).

4. Discussion

In charge of co-translational membrane/secretory protein targeting, SRP is conserved in all living things (Kellogg et al., 2021). Prokaryotes S. mutans, E. coli, B. subtilis, and eukaryotes S. cerevisiae have all been discussed in relation to the SRP pathway (Prinz et al., 2000; Hasona et al., 2005; Hasona et al., 2007; Phillips and Silhavy, 1992; Wickstrom et al., 2011). As of right currently, there is no information available regarding the SRP route for LAB. The SRP pathway components Ffh and FtsY of Lactobacillus lactis and S. mutans were predicted to have comparatively higher sequence identities using bioinformatic analysis. Additionally, similar operon structures containing the ftsY were discovered in Lactobacillus lactis and S. mutans (Hasona et al., 2005), suggesting a close phylogenetic relationship between the Ffh and FtsY from Lactobacillus lactis and S. mutans. According to Stackebrandt and Teuber (1988), the genus Lactococcus diverged from dairy streptococci in order to distinguish themselves from pathogenic streptococci. As a result, L. lactis shares a closer evolutionary relationship with S. mutans than B. subtilis and E. coli. According to earlier research, S. mutans is not killed by removing Ffh or FtsY because the insertase YidC2 may partially overlap the SRP pathway's activity (Mishra et al., 2019).

Proteins involved in glycolysis and pyruvate conversion, as the fructose- 1,6-diphosphate aldolase, 1-phosphofructokinase and L-lactate dehy- drogenase were decreased in the two mutants (Table 4). The results obtained here were consistent with those observed in S. mutans, as Hasona discovered that the levels of glycolytic enzymes decreased in the SRP mutants under non-stress conditions (Hasona et al., 2007). Inter- estingly, several proteins of ABC transporter and PTS system were found upregulated in the Δffh and ΔftsY, which might be a compensation in case of emergency. We also discovered the lactate concentration of the WT reached its peak at 6 h and gradually decreased under aerobic respiration condition, which should be a result of the conversion of lactate to acetate (Gaudu et al., 2002; Duwat et al., 2001). Similarly, the performances of impaired aerobic respiration were associated with the decreased level of NADH dehydrogenase, an essential composition of the electron transfer chain.

Resistance to acid stress is regarded as a generalized competence of LAB as a large amount lactate is produced during sugar fermentation. To cope with acid stress, LAB have developed various resistance mecha- nisms, such as proton pump F0F1-ATPase to expel intracellular H+, glutamate decarboxylase, arginine deaminase and urease (Guan and Liu, 2020). We found that the ffh and ftsY were transcriptionally upregulated corresponding to the acid challenge treated upon L. lactis NZ9000, while disruption of the Ffh or FtsY resulted in more acid sensitivity compared with the WT. Proteomic analysis showed that the Δffh and ΔftsY had the reduced levels of F0F1-ATPase and glutamate decarboxylase, probably yielding acid sensitive phenomena.

SRP-dependent protein targeting was firstly identified in mammalian cells, and subsequently in several bacteria forty years ago (Lauffer et al., 1985; Ro¨misch et al., 1989). However, the essentiality of SRP remains yet to be understood in L. lactis. This work revealed that the SRP is not essential in L. lactis, which is an economically important LAB and also serves as a research model for Gram-positive bacteria. Experimental evidence suggested that disruption of

the key SRP pathway components Ffh or FtsY resulted in impaired industrial natures including fermenta- tive and respirative metabolism as well as acid resistance. These phe- nomena were closely linked to increased chaperones and reduced membrane proteins as pointed by proteomic approach. Collectively, this study contributed to our understanding of the effects of the dispensable.

Our sequence retrieval revealed that L. lactis also included two copies of insertase (LLNZ_00755 and LLNZ_02780), which shared sequence homologies with S. mutans (Fig. S5A and B). Further research is necessary to determine the function of these copies. Thus, the identical genetic makeup of insertase and Ffh and FtsY suggested that the Ffh and FtsY requirement in L. lactis would be as little as that in S. mutans. Since the resulting mutant strains of Lactobacillus lactis Δffh and ΔftsY were varied, this hypothesis was validated by successfully introducing a gene mutation in the ffh and ftsY of Lactobacillus lactis NZ9000. In L. lactis, experimental data is still needed to determine if the putative insertases are replacing the SRP route. Furthermore, we were unable to cause multiple mutations in the ffh and ftsY genes, suggesting that L. lactis would not be able to withstand the simultaneous disruption of both Ffh and FtsY.

One important filter for dairy industrial starting is the acidification activity of Lactobacillus lactis (Coelho et al., 2022). Lactate is produced by L. lactis by fermenting soluble sugar in milk or broth. In this case, the Δffh and ΔftsY shown SRP route on L. lactis's industrial importance. Future studies on industrial strain screening and the mechanisms driving the strain's evolution during genomic degradation will be made easier by the information.

5. Conclusion

While disruption of the Ffh or FtsY resulted in poor growth, sugar metabolism, and acid sensitivity, the preserved SRP route is not necessary for L. lactis to survive. In conclusion, the dispensable SRP pathway may help keep L. lactis's metabolism in balance and help it withstand acid stress.

Declaration of competing interest

The authors declare that they have no competing interests or per- sonal relationships that could have influenced the work reported in this work.

Data availability

Data will be made available on request.

Acknowledgements

The authors affirm that none of their personal relationships or conflicting interests might have influenced the work described in this publication.

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Table 2
Primers used in this study.

Primers	Sequence (5′-3′)
FtsY-DSBF	AAACGGTGCTATTGACCAATTAGTTGAATG
FtsY-DSBR	AAAACATTCAACTAATTGGTCAATAGCACC
Ffh-DSBF	AAACTACCGGAAAACCTATTAAATTTACAG
Ffh-DSBR	AAAACTGTAAATTTAATAGGTTTTCCGGTA
ssDNA-ffh	
	GGTAGAAAACTTCAAGGTCG
GTTAATTTTTCACCAGTTCATCAAAATTTAATAGGTTTTCCGGTAATTTCA	
CGAATTGAT	ssDNA-ftsY
	ACCAGCTGGTTTTGTGACTAC
TTCAACGCCTGAACGATTTCAATGGTCGTTGTTTTACCAACACCATTTAC	
CCCAACAAA	Ffhcexu-F
	GATGAAGGAACGTCTGAAA
AACC	
Ffhcexu-R	GCATTTGTGACATTCCTGGAATC
FtsYcexu-F	TCGGTGTGGATTTGGCAATGGA
FtsYcexu-R	CCGAACCGTCAAGTTTAGTCA
ffhdl-F	TTGGGAGAACAACTTGACAT
ffhdl-R	CCTGCCGTATCAATCAACAC
ftsYdl-F	GGAAAGAAGAAACAAGAGGA
ftsYdl-R	CTGGCTTTTCAGTTACCTCA
16S-F	GTGTAGCCCAGGTCATAAGG
16S-R	CGTGTCGTGAGATGTTGGGT

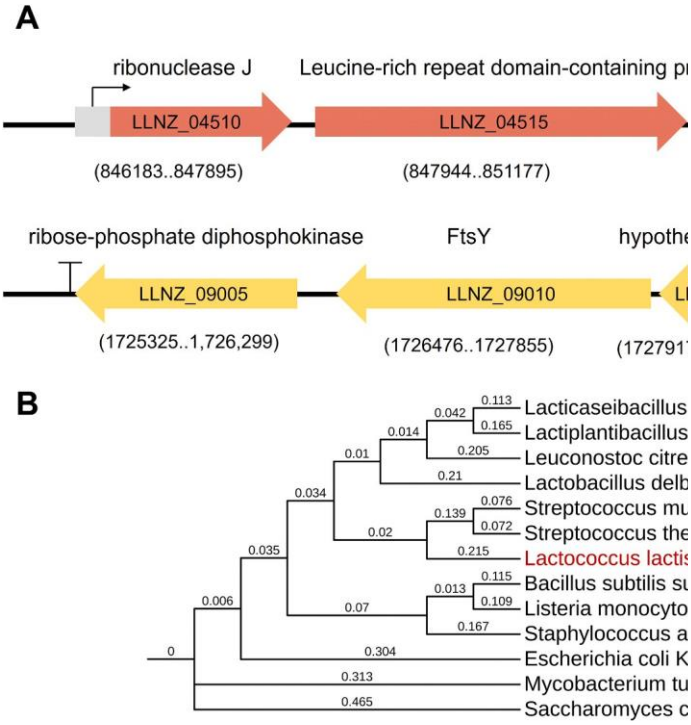

Fig. 1. Sequence analysis of the *ffh* and *ftsY* of *L. lactis* NZ9000. (A) Genetic structure prediction of the *ffh* and *ftsY* in the genome (B) Phylogenetic tree analysis of the tandem sequences of the Ffh and FtsY.