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RESEARCH ARTICLE

Comparative Genomics and Pangenome Analysis 0f *L. Paracasei* ATA-LTC-Lp121000 Strain: A Potential Probiotic from Türkiye

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Comparative Genomics and Pangenome Analysis of L. Paracasei ATA-LTC-Lp121000 Strain: A Potential Probiotic from Türkiye

SUMMARY

The isolation of novel Lacticaseibacillus strains with robust safety validation, technological stability, and specialized metabolic capabilities is crucial for advancing functional food and precision pharmabiotic industries. Since they are generally accepted as safe, many L. paracasei strains are suitable for use as probiotics. The aim of our study is to investigate the probiotic potential of the L. paracasei ATA-LTC-Lp121000 strain through in-depth genomics and pan-genomic analyses analyses of this strain against other closely related strains. A multi-dimensional genomic workflow was executed on a novel Lacticaseibacillus isolate. Intraspecific pangenome dynamics, genomic stability, safety markers, and secondary metabolome signatures, including Carbohydrate-Active enZymes (CAZymes) and ribosomally synthesized and post-translationally modified peptides (RiPPs) were mapped across 30 distinct strains of the same species. Genotypic profiling verified the absence of transmissible antimicrobial resistance (AMR) genes and virulence factors. Pangenome mapping revealed an open pangenome architecture. L. paracasei ATA-LTC-Lp121000 isolate potentially demonstrated good chromosomal stability driven by low density of mobile genetic elements (MGEs), specifically sparse IS30, IS5, and IS3 insertion sequences. Functional exploration revealed a rich enzymatic profile dominated by Hydrolase (GH1) and Glycoside Hydrolase families (GH13, GH25, and GH29, paired with RiPP biosynthetic gene clusters (BGCs). The L. paracasei strain ATA-LTC-Lp121000 is a good candidate for a probiotic because it has a relatively stable genomic structure and does not contain any antibiotic resistance genes or virulence factors. All these characteristics make it a safe, predictable, and ideal candidate for probiotics and food formulations. The phenotypic characteristics of this strain and its benefits as a probiotic should be tested experimentally.

Keywords: *Lacticaseibacillus paracasei ATA-LTC-Lp121000, probiotics, pangenomes, comparative genomics, RiPP biosynthetic gene clusters*

L. paracasei ATA-LTC-Lp121000 Suşunun Karşılaştırmalı Genomik ve Pangenom Analizi: Türkiye'den Potansiyel Bir Probiyotik

ÖZ

Güçlü güvenlik doğrulaması, teknolojik kararlılık ve özel metabolik yeteneklere sahip yeni Lacticaseibacillus suşlarının izole edilmesi, fonksiyonel gıda ve hassas farmabiyotik endüstrilerinin gelişmesi açısından hayati önem taşımaktadır. Genel olarak güvenli kabul edildikleri için, birçok L. paracasei suşu probiyotik olarak kullanıma uygundur. Çalışmamızın amacı, L. paracasei ATA-LTC-Lp121000 suşunun probiyotik potansiyelini, bu suşun derinlemesine genomik analizleri ve diğer yakından ilişkili suşlarla karşılaştırmalı pan-genomik analizleri yoluyla araştırmaktır. Yeni bir Lacticaseibacillus izolatı üzerinde çok boyutlu bir genomik iş akışı uygulanmıştır. Tür içi pangenom dinamikleri, genomik stabilite, güvenlik belirteçleri ve karbonhidrat-aktif enzimler (CAZymes) ile ribozomal olarak sentezlenen ve post-translasyonel olarak modifiye edilmiş peptitler (RiPPs) dahil olmak üzere ikincil metabolom imzaları, aynı türün 30 farklı suşu üzerinde haritalanmıştır. Genotipik profilleme, bulaşıcı antimikrobiyal direnç (AMR) genlerinin ve virülans faktörlerinin yokluğunu doğruladı. Pangenom haritalaması, açık bir pangenom mimarisi ortaya çıkarmıştır. L. paracasei ATA-LTC-Lp121000 izolatı, özellikle seyrek IS30, IS5 ve IS3 insersiyon sekansları olmak üzere, düşük mobil genetik eleman (MGE) yoğunluğu sayesinde iyi bir kromozomal stabilite potansiyeli sergilemiştir. Fonksiyonel araştırmalar, RiPP biyosentetik gen kümeleriyle (BGC'ler) eşleşen Hidrolaz (GH1) ve Glikozit Hidrolaz ailelerinin (GH13, GH25 ve GH29) baskın olduğu zengin bir enzimatik profil ortaya çıkarmıştır. L. paracasei ATA-LTC-Lp121000 suşu, nispeten kararlı bir genomik yapıya sahip olması ve herhangi bir antibiyotik direnç geni veya virülans faktörü içermemesi nedeniyle probiyotik için iyi bir adaydır. Tüm bu özellikleri, onu probiyotikler ve gıda formülasyonları için güvenli, öngörülebilir ve ideal bir aday haline getirmektedir. Bu suşun fenotipik özellikleri ve probiyotik olarak sağladığı faydalar deneysel olarak test edilmelidir.

Anahtar Kelimeler: *Lacticaseibacillus paracasei ATA-LTC-Lp121000, probiyotikler, pangenomlar, karşılaştırmalı genomik, RiPP biyosentetik gen kümeleri*

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INTRODUCTION

Lacticaseibacillus paracasei is a Gram-positive, non-spore-forming, facultative anaerobic, rod-shaped microorganism within the Lactic Acid Bacteria (LAB) group. As a result of a comprehensive taxonomic revision in 2020, the species was reclassified into the genus Lacticaseibacillus (Zheng et al., 2020). There are two subspecies within this species: subsp. paracasei and subsp. tolerans. *L. paracasei* is an ecologically versatile, commonly found in the human gastrointestinal tract, oral cavity, and vaginal microbiome, as well as in fermented dairy products, pickles, fermented meat products, and plant-based matrices (Balzaretti et al., 2015; Cai, Thompson, Budinich, Broadbent, & Steele, 2009; Smokvina et al., 2013). Since they can live in a wide variety of environments, they are able to adapt to different environmental factors.

Since they are generally accepted as safe, many *L. paracasei* strains are suitable for use as probiotics. They are widely used in the food industry as fermentation starters for the manufacturing of traditional products such as kefir and cheese (Bettera et al., 2023; Kiousi et al., 2022). *L. paracasei* HA-196 has been used as a probiotic to reduce symptoms of irritable bowel syndrome (Lewis et al., 2020), while *L. paracasei* HII01 has been used as a probiotic for patients with type 2 diabetes (Toejing, Khampithum, Sirilun, Chaiyasut, & Lailerd, 2021). The probiotic potential of *L. paracasei* strains is directly related survivalism in gastrointestinal tract, adhere to epithelial cells, inhibition of pathogen colonization, and immune system modulation (Jankowska, Laubitz, Antushevich, Zabielski, & Grzesiuk, 2008; Lim & Ahn, 2012; Ouwehand, Tölkkö, Kulmala, Salminen, & Salminen, 2000; Styriak, Nemcová, Chang, & Ljungh, 2003).

L. paracasei lowers surrounding pH by producing organic acids such as lactic acid, acetic acid, and succinic acid, thereby inhibiting pathogenic bacteria growth. In addition, through the bacteriocins they produce via RiPP-like gene cluster, they inhibit pathogens such as *Escherichia coli* and *Staphylococcus aureus*. For that reason, it is essential to determine biosynthetic gene clusters form genomes.

The *L. paracasei* ATA-LTC-Lp121000 strain is a potential probiotic isolated from an animal stool sample in Igdir, Türkiye by ATA Biotechnology Pharmaceutical Industry and Trade Ltd. Sti. The whole genome sequence of the strain has been compiled and uploaded to the NCBI biosample database under the BioProject accession number PRJNA1092961 with assembly number of GCF_038025045.1. Although the genome annotation for this strain has been completed, comparative genomics and pan-genome analyses have not been performed. Furthermore, detailed genomic analyses of the *L. paracasei* ATA-LTC-Lp121000 strain, as well as analyses of factors that could hinder its use as a probiotic—such as ARGs and virulence factors—and beneficial pathways such as bioavailability have not been studied.

The aim of our study is to investigate the probiotic potential of the *L. paracasei* ATA-LTC-Lp121000 strain through in-depth genomic analyses and to perform computational comparative genomic and pan-genomic analyses of this strain against other closely related strains.

MATERIALS AND METHODS

Genome selection

To perform comparative genomic analyses, 1176 genomes of the species *L. paracasei* were downloaded from the NCBI genome (https://www.ncbi.nlm.nih.gov/home/genomes/) database on August 6, 2026. Table S1 lists the accession numbers and other information for the selected genomes. The quality of the obtained genomes was assessed using the CheckM2 v1.1.0 program with default parameters based on completeness and contamination rates (Chklovski, Parks, Woodcroft, & Tyson, 2023). Genomes that were assembled at the complete genome level, had a contamination rate of less than 5.0%, and a completeness rate of more than 90.0% were included in the study with *L. paracasei* ATA-LTC-Lp121000

(GCF_038025045.1). Unless otherwise specified, default parameters were used in the bioinformatics analysis. Phylogenetic analyses of *L. paracasei* genomes were performed using genome sequences with the pyANI with the BLAST algorithm (ANIb) (Pritchard, Glover, Humphris, Elphinstone, & Toth, 2015).

Pan-genomics and core genome analysis

Pangenome analyses of *L. paracasei* genomes were conducted using the workflow recommended by the developers of the Anvi'o v8 program (Eren et al., 2015). Briefly, reads shorter than 2,500 bp were first removed. Subsequently, the profile of the *L. paracasei* genomes was generated using the `anvi-gen-contigs-database` command. First, open reading frames (ORFs) in the genomes were identified using the Prodigal program (Hyatt et al., 2010). Genome annotation was performed using anvio-run commands leveraging the HMMS (Lee, 2019) with NCBI-COGS (Tatusov, Galperin, Natale, & Koonin, 2000), KEGG-KOFAMS (Aramaki et al., 2020), and Cazymes databases (Drula et al., 2022), and the results were added to the genome databases. Subsequently, a pan-genome analysis was performed using the `anvi-pan-genome` command. The average nucleotide similarity between genomes was calculated using the `anvi-compute-genome-similarity` command with PyANI. The rarefaction curves for the pan-genome were generated using the `anvi-compute-rarefaction-curves` command. Finally, the resulting pan-genome was visualized using the `anvi-interactive` command, and a pan-genome graph was plotted.

Genes found in all genomes are designated as core genes. Genes found in only one genome are designated as singletons. Genes not represented in all genomes have been classified as accessory genes. Shell genes and cloud genes are components of accessory genes. For shell genes, genes found in 20% to 83.33% (6 to 25 genomes) of strains were selected. Genes observed in less than 16.67% (5 genomes) of genomes have been defined as cloud genes. Shell genes and cloud genes were identified by filtering them in the Anvi'o program (Eren et al., 2015).

Antibiotic resistance, virulence factors and mobile genetic element genes detection

Antibiotic resistance genes and virulence factors were identified using the ABRicate v1.4.0 program with default parameters (Seemann, 2016). The ABRicate program incorporates multiple databases and is able perform analysis across all those databases. All *L. paracasei* genomes were searched against the CARD (2026-Apr-3) (Alcock et al., 2023), NCBI AMRFinderPlus (2026-Apr-3) (Feldgarden et al., 2021), ARG-ANNOT (2026-Apr-3) (Gupta et al., 2014), Resfinder (2026-Apr-3) (Bortolaia et al., 2020), MEGARES (2026-Apr-3) (Bonin et al., 2023), PlasmidFinder (2026-Apr-3) (Carattoli et al., 2014), VFDB (2026-Apr-3) (Chen, Zheng, Liu, Yang, & Jin, 2016), and VICTORS (2026-Apr-3) (Sayers et al., 2019) databases. Mobile genetic elements were predicted using the MOB-suite v3.1.9 (Robertson & Nash, 2018).

Metabolic pathways and carbohydrate-active enzymes, secondary metabolite biosynthesis gen cluster analysis

Secondary metabolite biosynthetic gene clusters in *L. paracasei* ATA-LTC-Lp121000 genome were identified using the bacterial version of antiSMASH v8.0.4 (Blin et al., 2025). The METABOLIC v4.0 (Zhou et al., 2022) was used to predict the metabolic and biogeochemical function profiles of *L. paracasei* genomes. Briefly, open-reading frames were identified using Prodigal (Hyatt et al., 2010). Gene functions were then annotated using Diamond (https://github.com/bbuchfink/diamond) BLAST against the KOFAM (Aramaki et al., 2020), pfam (Mistry et al., 2021), MEROPs (Rawlings, Barrett, & Bateman, 2010), TIGRfam (Haft, Selengut, & White, 2003), dbCan2 (Zhang et al., 2018), and custom metabolic HMM databases (Zhou et al., 2022). From the results, a presence/absence table of KEGG identifiers and a presence/absence table consisting of KEGG modules and KEGG module steps were generated. Table for

carbon-active enzymes was generated from dbCan2 results. The phylogenetic tree was drawn using ITOL (https://itol.embl.de).

RESULTS

Genome analysis

The *L. paracasei* ATA-LTC-Lp121000 genome is a scaffold-level genome consisting of 99 contigs and is 2,990,200 bp in length (Table 1, Figure 1.). A total of 2,938 genes were identified, 1,458 of which have been assigned to the KEGG database. The cas3_Type I protein and one CRISPR locus was detected. A total of 102 genes belonging to Lactobacillus phages have been identified. As a result of an analysis of the KEGG database, 1,458 genes were functionally annotated. It was observed that these genes are associated with 201 pathways and that there are 33 complete pathways. These pathways are related to carbohydrates, lipoprotein, nucleotide metabolism, energy metabolism, and glycan metabolism. Additionally, complete C10-C20 isoprenoid biosynthesis pathway is present. *L. paracasei ATA-LTC-Lp121000* strain was found to belong to the same clade as *L. paracasei* D11 strain based on ANIb similarity (Figure 2.). *L. paracasei ATA-LTC-Lp121000* strain does not contain any harmful genes, such as antibiotic resistance genes or virulence factors based on ABRICATE analysis.

Table 1. General characteristics of *L. paracasei* ATA-LTC-Lp121000 genome assembly

Genome	
Assembly level	Scaffold
number of contigs	99
Length (bp)	2,990,200
GC content	46.5
Total genes	2938
Coding DNA (%)	94.96
Protein-coding genes	2790
Pseudogenes	82
Genes assigned to KEGG	1458
Complete KEGG module	33
Number of KEGG pathways	201
rRNA genes	4
tRNA genes	67
CRISPR locus	1
Cas proteins	cas3 Type I

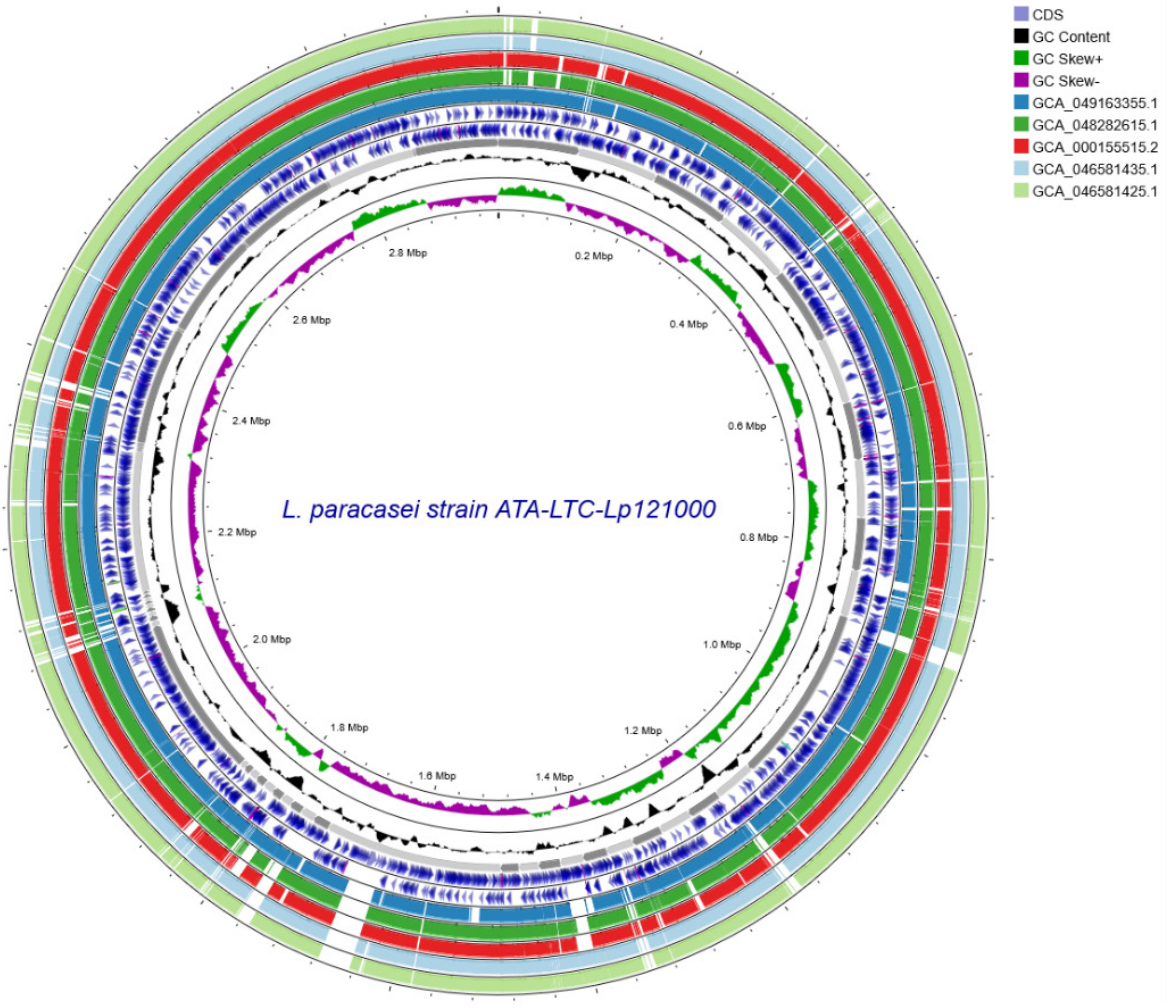


Figure 1. Circular genome map and comparative genomics of *L. paracasei* ATA-LTC-Lp121000 strain generated by Proksee Server. Blue arrows indicate CDSs, while contigs are shown in gray. GC content is displayed in black. Positive GC skew is shown in green, and negative GC skew is shown in purple. The outer rings show nucleotide BLAST similarity scores for the strains with the highest ANIb similarity. The accession numbers of the genomes are listed in the legend

Lactic Acid Bacteria (LAB) produces bacteriocins via RiPP-like gene clusters to inhibit other pathogenic bacteria (Alvarez-Sieiro, Montalbán-López, Mu, & Kuipers, 2016). Clusters of secondary metabolite genes were identified in the *L. paracasei* ATA-LTC-Lp121000 genome using the antiSMASH program. *L. paracasei* ATA-LTC-Lp121000 has three biosynthetic gene clusters (Figure 3.). Two of them belong to RiPP-like gene clusters which can produce various secondary metabolites including bacteriocins. Other gen cluster belong to NRPS-like gene cluster which can produce terpene-precursor.

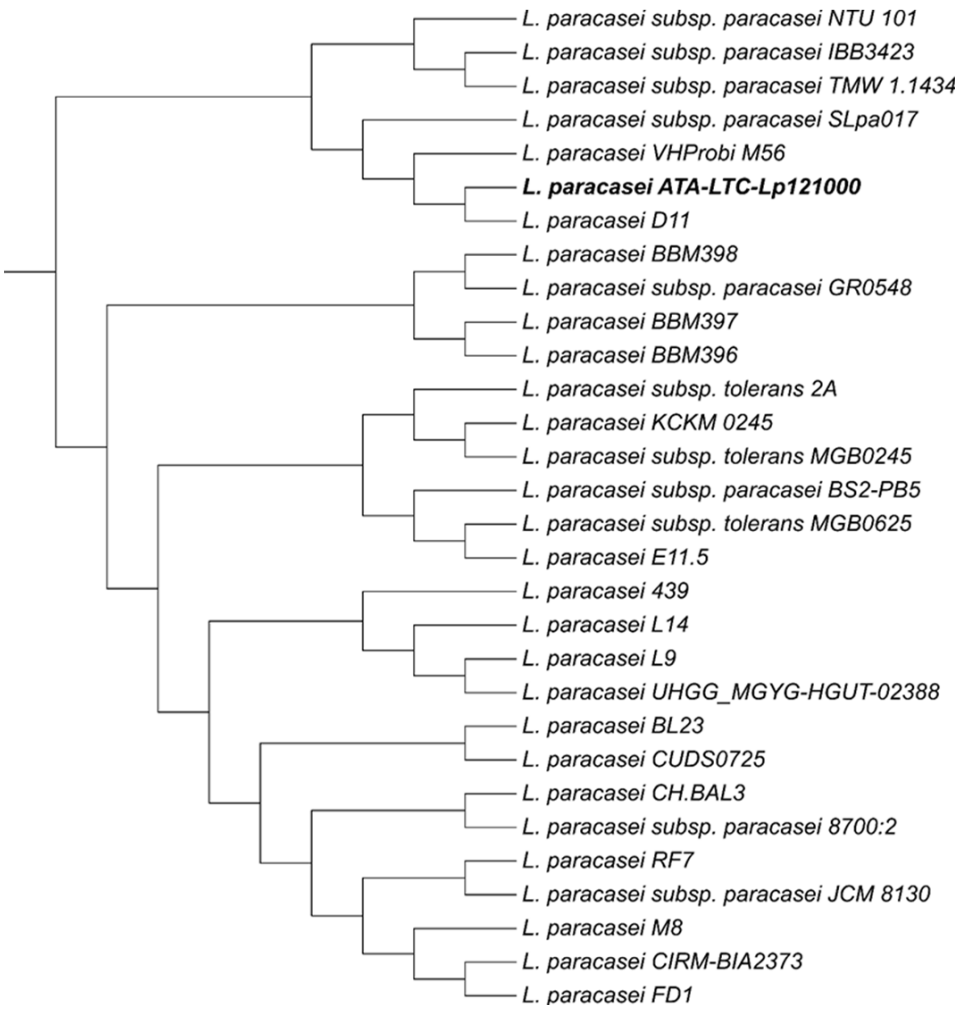


Figure 2. Phylogenetic tree of 30 *L. paracasei* strain used this study based on ANIb similarity

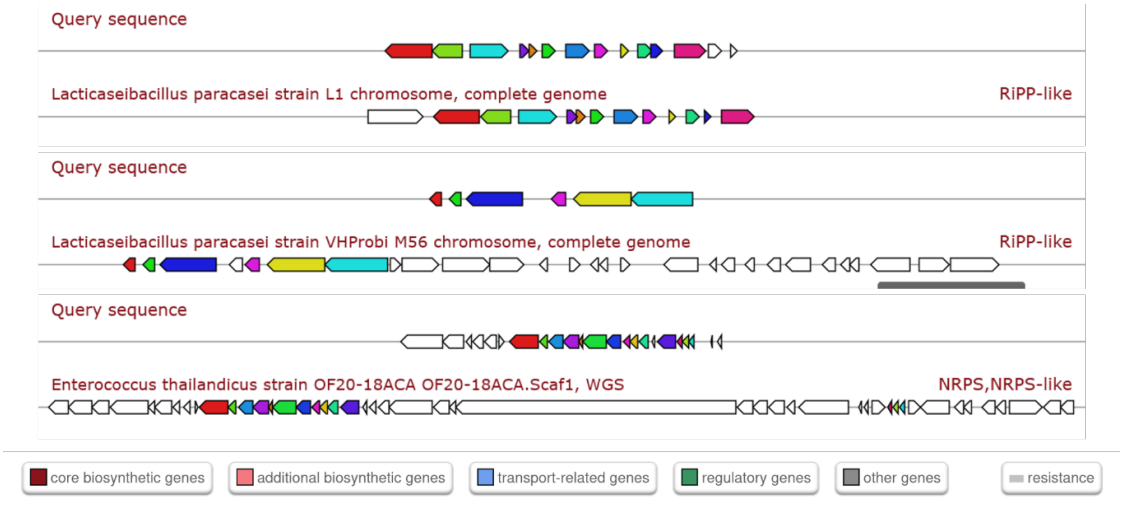


Figure 3. A representative graph of biosynthetic gene clusters identified in *L. paracasei* ATA-LTC-Lp121000 strain via search on the Antismash server

Genome selection for pangenome analysis

A total of 1176 *L. paracasei* genomes were downloaded from the NCBI genome database on August 6, 2026. Based on the quality assessment of the genomes, their completeness ranges from 22.59% to 99.09%. The contamination rate in the genome ranged from 0.45% to 18.43%. A total of 244 genomes were complete, 26 genomes were chromosomal levels, and 906 of them were assembled at the scaffold and contig level (Figure 4.). The number of genes identified in *L. paracasei* genomes ranges from 6,644 to 1,288. The number of genes identified in complete genomes is higher than in genomes at the scaffold and contig levels (Figure 4.). The average genome size is 2,966,435.34± 247,714.02 bp, and the GC content is approximately 46.4%. Detailed metadata for the selected genomes is provided in Supplementary Table S2. A total of 29 genomes meeting the criteria of a contamination rate lower than 5.0% and a completeness rate greater than 90.0%, assembled at the scaffold genome level *L. paracasei* ATA-LTC-Lp121000 strain were included in the study for pangenome and comparative genomes analysis.

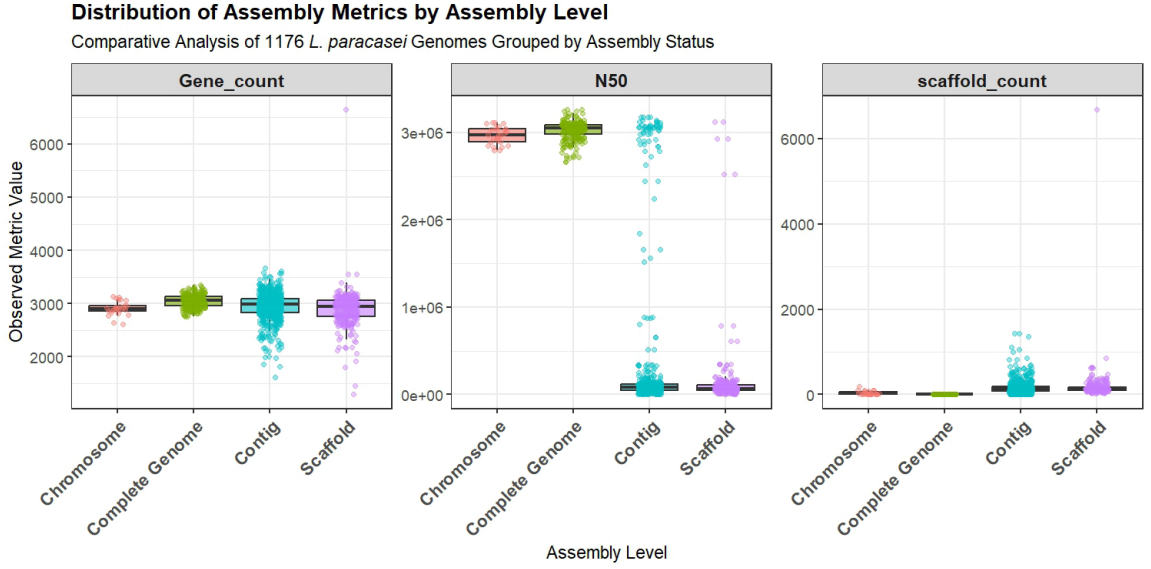


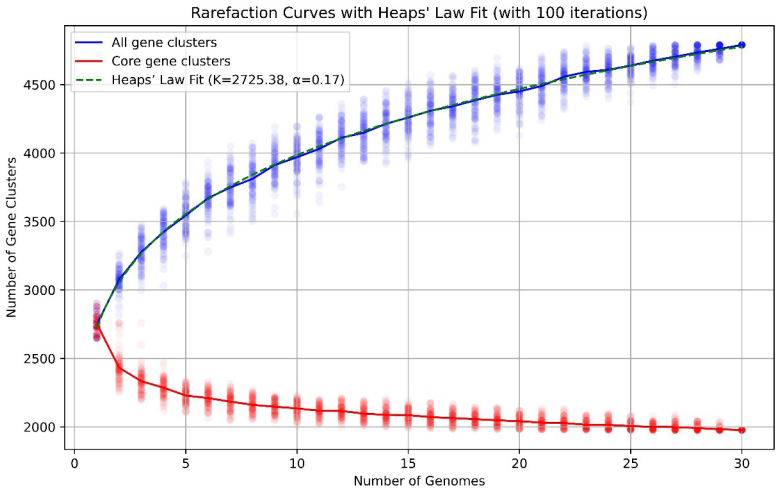
Figure 4. Distribution of assembly matrix based on assembly level of 1176 *L. paracasei* genomes

Pan-genomics and core genome analysis

Pangenome studies are a less bias-prone method than comparative individual genome analyses. They are used for species and variant identification, genotyping, and accurate phenotype identification (Matthews, Watson-Haigh, Burton, & Sheppard, 2024; The Computational Pan-Genomics, 2018). Pangenome analyses of the 30 *L. paracasei* genomes were performed through BLAST using Anvio v8 (Eren et al., 2015). In addition, the similarity between genomes was calculated using the PyANI. The alpha value was calculated as 0.17 for the 30 *L. paracasei* genome using Heaps' law (Figure 5A.). Since this alpha value is less than 1, it has been classified as an open pangenome. In open pangenomes, new gene clusters are added to the pangenome as new genomes are incorporated showing very broad gene pools for species. A total of 89,024 genes were identified within 4,790 gene clusters in the 30 *L. paracasei* genomes. Genes represented at least once across all genomes were identified as core genomes. A total of 48,600 genes were identified within 1,620 gene clusters in the core genome. These genes are involved in processes ranging from carbohydrate metabolism to energy metabolism, and from lipid metabolism to nucleotide and amino acid metabolism which are highly conserved due to high selective pressure.

Genes present in a subset of the *L. paracasei* genomes have been classified as accessory genomes. Shell genes and cloud genes are pieces of accessory genes. For shell genes, genes found in 20% (6 genomes) to 83.33% (25 genomes) of strains were selected. Genes observed in less than 16.67% (5 genomes) of genomes have been defined as cloud genes. A total of 1648 gene

clusters, comprising 37,457 genes, were identified as shell genes. These genes are less conserved and reflect the evolutionary history of the strains as well as their distinct phenotypic characteristics. Remaining 1522 gen clusters with 2,967 genes are designated as cloud genes which represent less than 6 genomes. Genes present in only a single genome are called singletons. In our 30 *L. paracasei* genomes-based pangenome, 837 singletons were identified (Figure 5B.). A total of 3 clusters were identified in the phylogenetic tree constructed based on ANIb similarities ranging from 98% to 100% among the genomes (Figure 5B.).



Phylogenetic Tree of L. paracasei 30 Genomes (1620 Single Copy Core Genes)

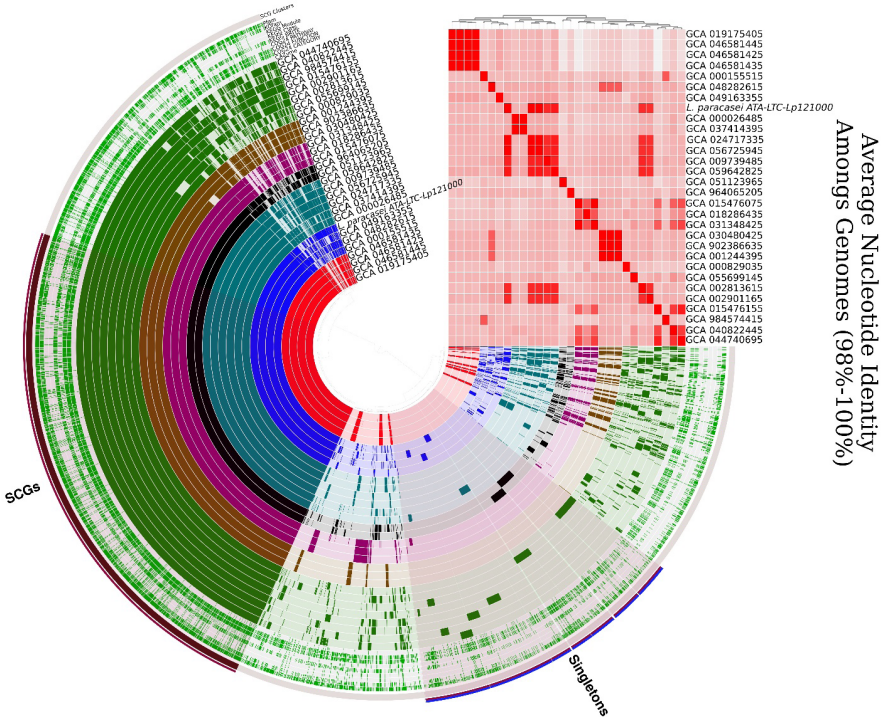


Figure 5. The pan-genome of *L. paracasei*. **A.** Rarefaction curve graph of Heaps' law fit. **B.** Each of the 4,790 gene clusters derived from the 30 genomes is represented by a circle composed of the 30 genomes. In the upper right corner, the genomes are arranged according to a phylogenetic tree constructed based on their ANI similarity and are color-coded by cluster. SCGs (single-copy gene clusters) and singletons are highlighted. C. Heatmap of ANIb similarity of genomes. Phylogenetic tree constructed based on their ANIb similarity. 98%

similarity was selected as minimal cut-off value for heatmap analysis

Antibiotic resistance, virulence factors and mobile genetic element genes detection

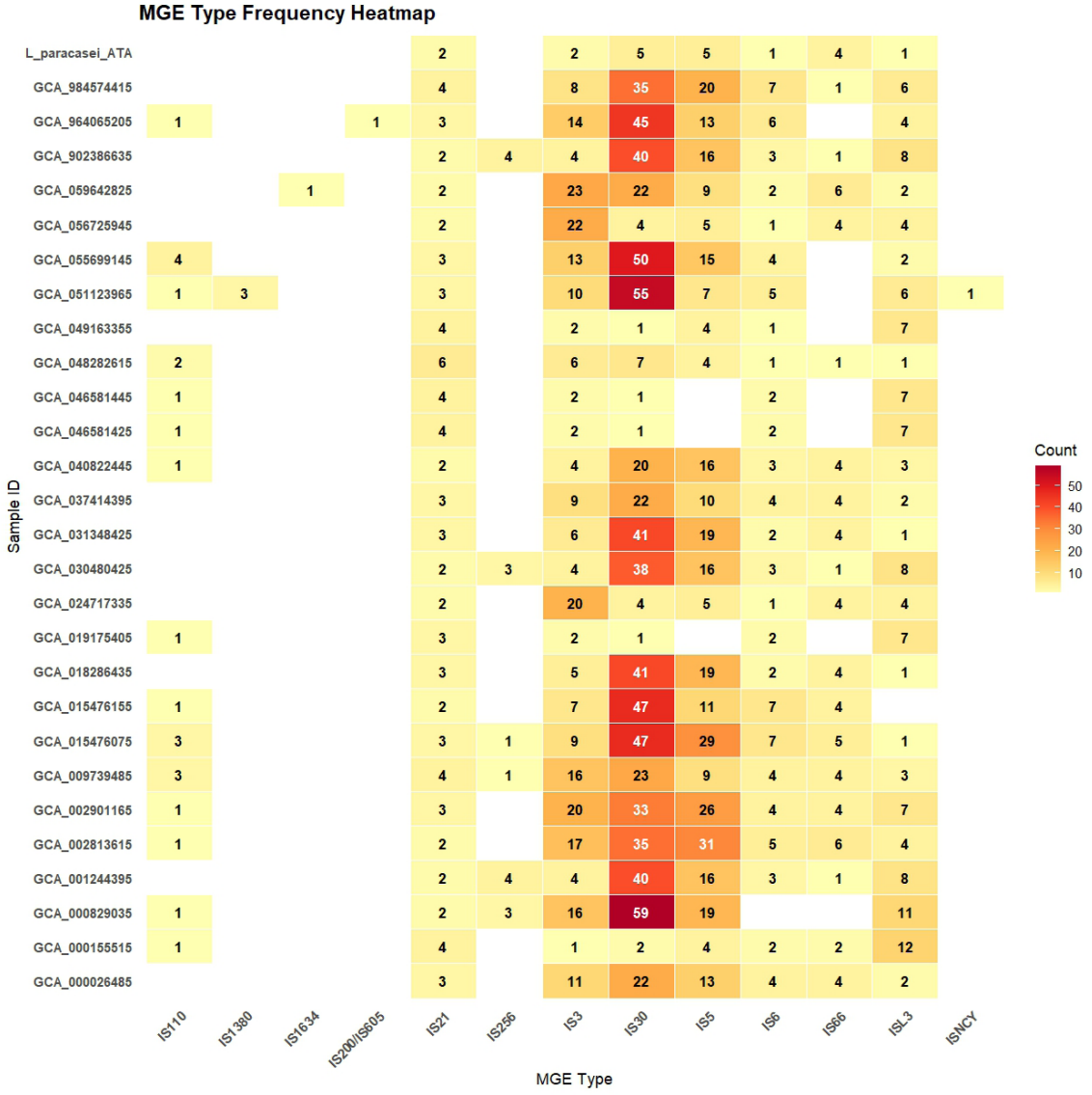
Antibiotic resistance genes and virulence factors were identified using the ABRicate v1.4.0 program (Seemann, 2016) with cut off value off 80% for both sequence identity and coverage. The ABRicate program incorporates multiple databases and is able perform analysis across all those databases. All selected *L. paracasei* genomes were searched against the NCBI AMRFinderPlus (Feldgarden et al., 2021), CARD (Alcock et al., 2023), ARG-ANNOT (Gupta et al., 2014), Resfinder (Bortolaia et al., 2020), MEGARES (Bonin et al., 2023), PlasmidFinder (Carattoli et al., 2014), VFDB (Chen et al., 2016), and VICTORS (Sayers et al., 2019) databases. As a result of the analyses, no ARGs or virulence factors were detected in any of the genomes.

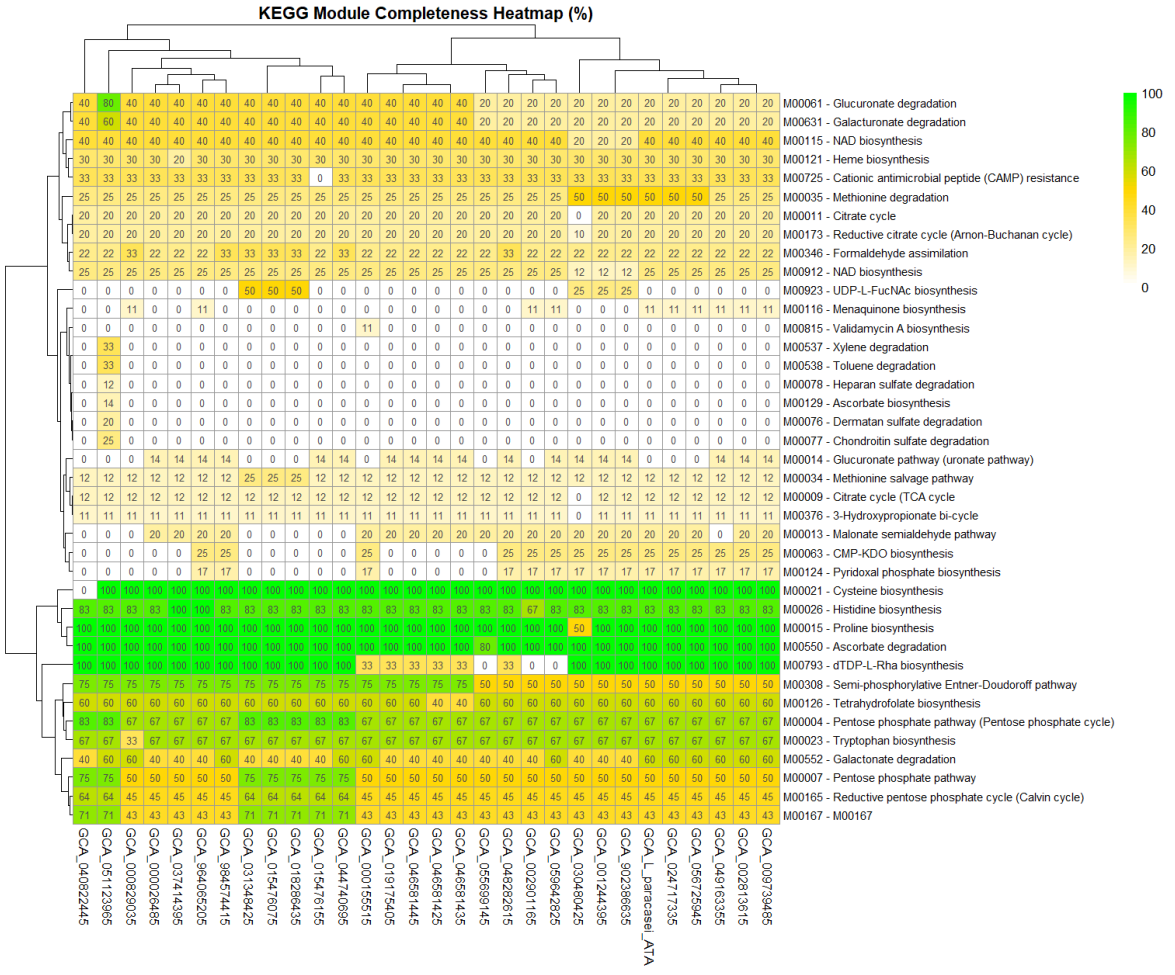
Mobile genetic elements are essential for the adaptation and evolutionary advancement of bacteria. Mobile genetic elements of pangenome were predicted using the MOB-suite v3.1.9 (Robertson & Nash, 2018). IS30, IS5, and IS3 were the most common IS types in the pangenome (Figure 6A.). While a high number of IS elements were detected in 30 strains, 10 strains, including the *L. paracasei* ATA-LTC-Lp121000, contained relatively fewer IS elements. The IS30 MGE, first identified in Escherichia coli K12, is 1,221 bp long and codes for a 383-amino-acid protein that functions as a transposase. IS30 is the most frequently detected MGE in the examined genomes. In fact, the copy number of IS30 reached as high as 59 copies in some strains. Five copies of IS30 were detected in the *L. paracasei* ATA-LTC-Lp121000 strain.

Metabolic pathways and carbohydrate-active enzymes

The METABOLIC v4.0 (Zhou et al., 2022) was utilised to predict the metabolic and biogeochemical function profiles of *L. paracasei* genomes as well as Cazymes profile. A total of 131 KEGG modules were assigned across the 30 strains included in the pangenome. All genomes possess a total of 22 complete KEGG modules including the Central carbohydrate metabolism, Purine metabolism, Pyrimidine metabolism, and Cofactor and vitamin metabolism modules. A heatmap showing the modules that differentiate the pangenome is presented in Figure 6B. While all genomes are similar in the amino acid, purine, and pyrimidine synthesis modules, the degradation of xenobiotics such as xylene and toluene is present in only one strain. *L. paracasei* ATA-LTC-Lp121000 is metabolically similar to the other strains. Hydrolase Family 1, the Glycoside Hydrolase Family (13, 25, and 29) is the most detected cazyme family in the pan-genome (Figure 6C.). The *L. paracasei* ATA-LTC-Lp121000

strain displays similar Cazyme profile to that of the pangenome.




KEGG Module Completeness Heatmap (%)
M00061 - Glucuronate degradation
M00631 - Galacturonate degradation
M00115 - NAD biosynthesis
M00121 - Heme biosynthesis
M00725 - Cationic antimicrobial peptide (CAMP) resistance
M00035 - Methionine degradation
M00011 - Citrate cycle
M00173 - Reductive citrate cycle (Arnon-Buchanan cycle)
M00346 - Formaldehyde assimilation
M00912 - NAD biosynthesis
M00923 - UDP-L-FucNAc biosynthesis
M00116 - Menaquinone biosynthesis
M00815 - Validamycin A biosynthesis
M00537 - Xylene degradation
M00538 - Toluene degradation
M00078 - Heparan sulfate degradation
M00129 - Ascorbate biosynthesis
M00076 - Dermatan sulfate degradation
M00077 - Chondroitin sulfate degradation
M00014 - Glucuronate pathway (uronate pathway)
M00034 - Methionine salvage pathway
M00009 - Citrate cycle (TCA cycle
M00376 - 3-Hydroxypropionate bi-cycle
M00013 - Malonate semialdehyde pathway
M00063 - CMP-KDO biosynthesis
M00124 - Pyridoxal phosphate biosynthesis
M00021 - Cysteine biosynthesis
M00026 - Histidine biosynthesis
M00015 - Proline biosynthesis
M00550 - Ascorbate degradation
M00793 - dTDP-L-Rha biosynthesis
M00308 - Semi-phosphorylative Entner-Doudoroff pathway
M00126 - Tetrahydrofolate biosynthesis
M00004 - Pentose phosphate pathway (Pentose phosphate cycle)
M00023 - Tryptophan biosynthesis
M00552 - Galactonate degradation
M00007 - Pentose phosphate pathway
M00165 - Reductive pentose phosphate cycle (Calvin cycle)
M00167 - M00167
GCA_040822445
GCA_051123965
GCA_000826035
GCA_000026485
GCA_037414395
GCA_964065205
GCA_964574415
GCA_031348425
GCA_015476075
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GCA_045581445
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GCA_045681435
GCA_055689145
GCA_048282815
GCA_002901165
GCA_059842825
GCA_030480425
GCA_001244395
GCA_902386635
GCA_L_paracasei_ATA
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GCA_049163355
GCA_002813615
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100
80
60
40
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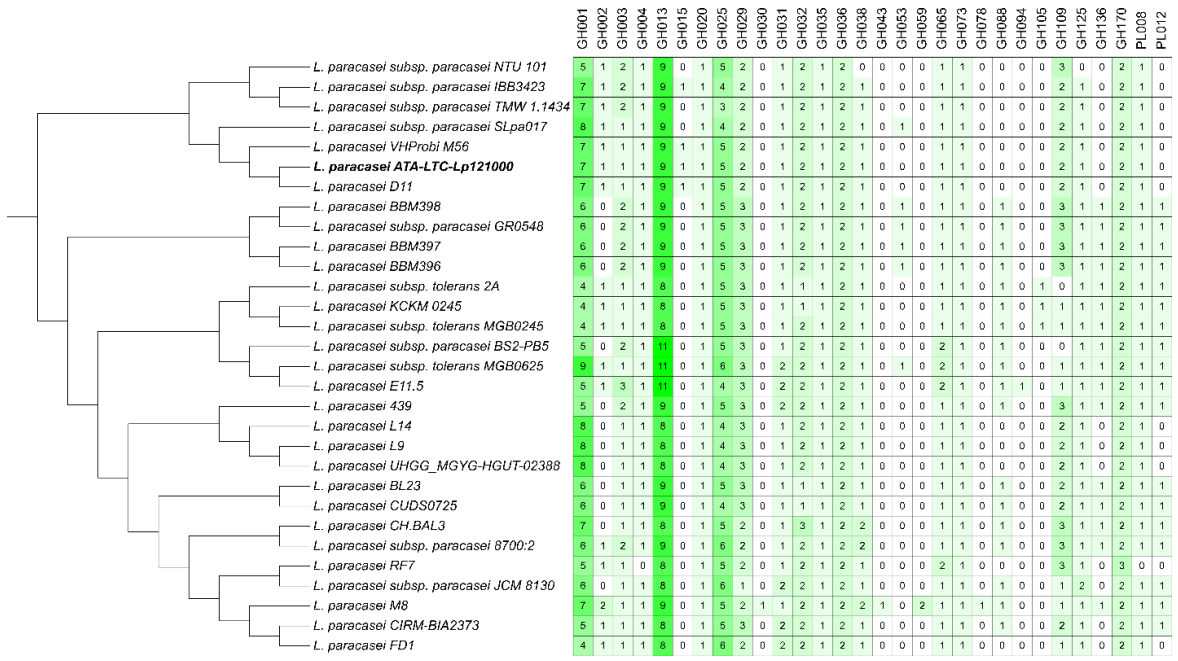

Figure 6. A. A heat map comparing mobile genetic elements. **B.** A heat map comparing the major metabolic pathways of *L. paracasei* ATA-LTC-Lp121000 with those of other strains in the pan-genome, based on KEGG annotation and METABOLIC analysis. **C.** Carbohydrate-active enzyme (CAZyme) profiles across genomes; dark green indicates a higher abundance of enzymes in the CAZyme family, while white indicates a lower abundance

DISCUSSION

Lactic acid bacteria (LAB) represent a cornerstone of functional microbiology, primarily due to their ubiquitous presence in traditional fermented matrices and the human gastrointestinal tract (Anumudu, Miri, & Onyeaka, 2024). The isolation of novel LAB strains from diverse biodiverse ecosystems, such as artisanal foods, breast milk, or mucosal niches, has emerged as a crucial frontier in medical biotechnology and personalized nutrition (Barman et al., 2025; Khushboo, Karnwal, & Malik, 2023; Lee, Choi, Song, & Cho, 2026).

Because the therapeutic and physiological efficacy of these microorganisms is highly strain-specific, discovering uncharacterized isolates provides a targeted strategy to address specific clinical pathologies where conventional commercial strains fall short (Khushboo et al., 2023; Mahrouk et al., 2026). While well-known probiotic strains such as *Lactobacillus acidophilus* LA-5 or *Lactobacillus rhamnosus* GG (LGG) are widely used for basic digestive and immune support, newly characterized indigenous strains within the *Lacticaseibacillus* genus often exhibit distinct probiotic profiles (Ghoshal et al., 2026). Overall, characterizing the biological diversity of wild *Lacticaseibacillus* isolates provides a fundamental basis for advancing preventive medicine and public health interventions.

From a biotechnological perspective, the new generation of Lacticaseibacillus isolates exhibit good functional properties compared to conventional industrial strains. Recent empirical evidence indicates that new wild-type isolates exhibit higher survival capacity under harsh gastrointestinal conditions characterized by low stomach pH and toxic bile salt concentrations, thereby enabling more efficient mucosal colonization (Barman et al., 2025; Khushboo et al., 2023). For example, newly identified *Lactiplantibacillus plantarum* strains (Zare, Aryaee, Mirdamadi, & Shirkhan, 2024) have demonstrated remarkable abilities to alleviate metabolic syndromes and restore the integrity of the

intestinal barrier. Similarly, newly isolated *Lactobacillus reuteri* strains (Mu, Tavella, & Luo, 2018) exhibit potent antagonistic effects against pathogens through the biosynthesis of reuterin and other low-molecular-weight antimicrobial compounds (Khushboo et al., 2023). Furthermore, newly characterized Lacticaseibacillus strains have drawn attention for their immunomodulatory effects, particularly in reducing the expression of proinflammatory cytokines and inhibiting *Helicobacter pylori* colonization in inflammatory bowel disease (IBD) (Lee et al., 2026). The unique antioxidant capacities, antidiabetic enzyme inhibitions (Won et al., 2021) (such as alpha-amylase inhibition), and macrophage-modulating profiles of these new isolates highlight their potential as specialized biotherapeutic agents (Lee et al., 2026; Mahrouk et al., 2026).

Apart from general effectiveness, the regulatory approval and commercial applicability of any new probiotic strain fundamentally depend on a careful safety assessment. In recent years, the European Food Safety Authority have issued strict guidelines requiring conclusive evidence that candidate strains do not pose a biosafety risk (EFSA) (Ziegler, Stark, Meier, & Brodmann, 2026).

The primary safety concern regarding the introduction of wild-type Lacticaseibacillus strains into the human gastrointestinal tract is the potential for horizontal gene transfer (HGT). If a probiotic strain carries antimicrobial resistance (AMR) genes or active virulence factors acquired via mobile genetic elements such as plasmids or transposons, these hazardous traits can be transferred to opportunistic pathogens in the gut microbiome (Mohammed, El-Said, Abd ElAal, & Kamal, 2024). Thus, verifying the genomic and phenotypic safety of a probiotic isolate is an essential requirement for the practical evaluation of any potential therapeutic or technological benefits.

The absence of virulence factors or antibiotic resistance genes in the *L. paracasei* ATA-LTC-Lp121000 genome indicates that it can be safely used as a probiotic in terms of lack of pathogenicity and the absence of ARG genes. The identification of strains that are both phenotypically and genotypically safe ensures that they will not contribute to the growing global crisis of antimicrobial resistance (Lee et al., 2026). Although *L. paracasei* ATA-LTC-Lp121000 is genotypically safe because it does not contain any ARG or VF, its phenotypic safety needs to be tested experimentally. Unlike many wild isolates that harbor innate or acquire resistance to critically important antimicrobials, this specific strain does not pose a risk of serving as a genetic reservoir for pathogens. Furthermore, the absence of virulence factors could indicate that the organism lacks the cellular mechanisms necessary to transition from a commensal state to an opportunistic pathogen, even in immunocompromised hosts (Mahrouk et al., 2026) however, experimental validation is required. Ultimately, good genomic safety profile makes the isolate an good candidate for probiotic application, minimizing regulatory challenges and ensuring better consumer safety (Barman et al., 2025; Loo et al., 2025).

The pronounced representation of the shell and cloud genes highlights the high level of strain-specific diversity among members of the same species. Importantly, comparative pangenome analysis revealed that our new isolate possesses a noticeably lower abundance of mobile genetic elements (MGEs)—including sparse distribution of IS30, IS5, and IS3 insertion sequences—compared to the species' broader pangenome reservoir. In a pangenome where some strains typically accumulate volatile single genes and cloud genes to an extreme degree at the expense of stability, our strain exhibits a relatively stabilized chromosomal architecture compared to others in pangenome. This structural protection could limit its involvement in intragenomic rearrangements, effectively minimizing the risk of spontaneous deletion, phenotypic drift, or fluctuations in the volatile accessory genome during successive generation cycles (Loo et al., 2025). This good stability may contribute to the strain remaining genetically stable during industrial production (Barman et al., 2025; Loo et al., 2025).

To evaluate the metabolic profile underlying the intraspecific diversity of 30 *Lacticaseibacillus* genomes, annotation mapping was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. The resulting KEGG pathway module heat map revealed uniformity across all 30 strains, indicating the presence of a good homogeneous metabolic core despite the variability observed in the structural accessory genomes. This functional clustering indicates that fundamental physiological frameworks—such as central carbohydrate metabolism, nucleotide biosynthesis, essential amino acid transformations, and cellular repair networks—are strictly conserved across the entire range of strains. The high degree of similarity observed in the heat map suggests that evolutionary pressures within the species have prioritized the conservation of these metabolic modules to maintain uniform basic survival strategies within host-dependent and environmental niches.

While structural pangenome mapping reveals high genetic variability in the accessory segments of sister strains, the KEGG heatmap confirms that this genomic fluidity does not lead to functional fragmentation or metabolic deviations. This metabolic homogeneity provides a good foundation for our new *Lacticaseibacillus* strain, which has a decrease in mobile genetic elements (MGEs) with lack of antibiotic resistance genes and virulence factors. This ensures that the strain could meet stringent regulatory requirements for predictable safety and consistent metabolic profiles, while its specialized secondary metabolome allows it to precisely deliver strain-specific health benefits without deviating from the established species-level metabolic signature (Lee et al., 2026; Loo et al., 2025).

Despite maintaining a highly stable and structured chromosomal core, our isolate retains metabolic versatility, as evidenced by its rich genomic potential of Carbohydrate-Active enZymes (CAZymes). From a probiotic perspective, the colonization success and metabolic efficacy of a *Lacticaseibacillus* strain are largely dictated by its capacity to decode and utilize complex environmental carbohydrates within host-dietary and mucosal niches. Within this framework, the high frequency of the Glycoside Hydrolase (GH) superfamily—specifically Families 1, 13, 25, and 29—serves as a vital indicator of specialized prebiotic assimilation and ecological fitness (Barman et al., 2025; Van Den Broek & Voragen, 2008). For instance, GH1 enzymes (β-glucosidases and β-galactosidases) enable the hydrolysis of plant-derived glycosides and lactose, while the GH13 family represents a versatile arsenal of α-amylases and pullulanases crucial for degrading complex starches and glycogen structures (Møller et al., 2012). This enzymatic repertoire could ensure that the strain can efficiently exploit non-digestible dietary fibers, giving it a distinct metabolic advantage over competing luminal microbes. However, the probiotic effect needs to be demonstrated experimentally.

Furthermore, the presence of specialized hydrolases like the GH25 and GH29 families could expands the strain's role from baseline digestion to mucosal crosstalk and pathogen exclusion. The GH25 family primarily encodes lysozyme-like enzymes that participate in controlled autolytic cell-wall remodeling necessary for growth, while acting as targeted biological weapons by degrading the peptidoglycan layers of competing Gram-positive pathogens (Sun, Zhao, Li, Kwok, & Zhang, 2025). Concurrently, the GH29 family, which comprises specialized α-L-fucosidases, allows the probiotic to cleave fucosylated glycans found abundantly in human milk oligosaccharides (HMOs) and the intestinal mucin layer (Sun et al., 2025). This specific fucosidase activity not only facilitates early-life gut colonization and long-term persistence along the mucosal barrier but also prevents the adherence of enterohemorrhagic pathogens that target identical glycan receptors. Consequently, an isolate featuring this synchronized array of GH1, GH13, GH25, and GH29 families represents a good candidate for functional food formulation (Møller et al., 2012; Sun et al., 2025).

Beyond verifying that the isolate lacks detrimental elements, maintains relative chromosomal stability, and exhibits high prebiotic versatility, their probiotic capability could be driven by its specialized secondary metabolome (Zhang et al., 2025). Computational genome mining of *Lacticaseibacillus* has revealed an abundance of RiPP biosynthetic gene clusters (BGCs) (Zhang et al., 2025). Unlike standard non-modified peptides, RiPP BGCs encode both precursor peptides with GG-motifs and peptidase-containing ATP-binding transporters (Fernandez-Cantos et al., 2023). These intricate structural architectures grant the resulting mature peptides, often termed bacteriocins, remarkable biophysical stability and extreme resistance against proteolysis within the hostile, enzyme-rich environment of the host gastrointestinal tract.

The primary ecological and clinical advantage of encoding RiPP-like clusters lies in the targeted antimicrobial deployment, frequently referred to as "molecular knives" (Wang et al., 2024; Zhang et al., 2025). Probiotics harboring active RiPP BGCs can synthesize antimicrobial peptides such as bacteriocins that suppress of opportunistic pathogens (Zhang et al., 2023). This localized pathogen antagonism not only facilitates the colonization and persistence of the producing *Lacticaseibacillus* strain within mucosal niches but also plays a vital role in resolving gut dysbiosis (Mills et al., 2025). Furthermore, contemporary probiogenomic insights indicate that certain gut-associated RiPP families participate actively in signaling, host immunomodulation, and intestinal barrier reinforcement by acting as quorum-sensing autoinducers that upregulate epithelial tight junction proteins or downregulate pro-inflammatory cascades in macrophage populations (Li & Rebuffat, 2020). Harnessing these specialized clusters allows for the rational design of pharmabiotics and next-generation functional foods capable of delivering precise, strain-specific health benefits. Although the *L. paracasei* ATA-LTC-Lp121000 genome contains RiPP-like gene clusters, the probiotic effects of the secondary metabolites produced by these gene clusters—such as the suppression of opportunistic pathogens, the resolution of gut dysbiosis, and anti-inflammatory effects—must be experimentally tested.

CONCLUSION

In conclusion, the multi-dimensional genomic evidence of the new *L. paracasei* ATA-LTC-Lp121000 isolate emphasizes its good potential good potential as a probiotic. Regarding clinical safety, structural potential genomic stability, and metabolic versatility, the strain meets regulatory standards on genomic level.

This study is limited to the genomic characteristics of the *L. paracasei ATA-LTC-Lp121000* strain. The results presented in this article and the conclusions drawn from comparative genomic analysis and bioinformatics findings. The results have not been validated experimentally or in vivo studies. The *L. paracasei* ATA-LTC-Lp121000 strain must be tested in experimental and animal models to determine whether it is safe as a probiotic and to demonstrate its beneficial effects.

Genotypic assay confirmed the absence of transferable antimicrobial resistance genes and virulence determinants, ensuring that it has low risk of acting as an epidemiological reservoir for genetic transfer within the host microbiota. Intraspecific pangenome mapping of 30 strains revealed an open pangenome structural architecture (1,620 core, 1,648 shell, 1,522 cloud, and 837 singletons); however, our isolate deviates favorably by exhibiting a lower density of mobile genetic elements, specifically within the IS30, IS5, and IS3 insertion sequence families. This low density of mobile genetic elements could reduce unpredictable phenotypic drift and may contribute downstream technological stability and sustainability during commercial upscaling. When paired with secondary metabolics and Cazyme metabolome including annotated Hydrolase and Glycoside Hydrolase families (GH1, GH13, GH25, GH29) for prebiotic synthesis and intact RiPP biosynthetic gene clusters for target pathogen exclusion—this *Lacticaseibacillus* strain appears as a good, safe, candidate as probiotics in functional foods, and precision pharmabiotics.

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AUTHOR CONTRIBUTION STATEMENT

HK: Conceptualization, Methodology, Investigation, Writing – Original Draft, Writing – Review & Editing.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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