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Whole-genome sequencing and *in vitro* probiotic characterization of *Pediococcus pentosaceus* ELAB 60WB isolated from fermented cherry tomatoes

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ABSTRACT

Pediococcus pentosaceus is known for its probiotic properties and is used in food fermentation. In this study, we performed *in vitro* probiotic characterization, genomic analysis, and safety analysis of the *P. pentosaceus* ELAB 60WB strain isolated from fermented cherry tomatoes. The strain exhibited antagonistic activity, survived exposure to gastric juice, and showed antibiotic activity *in vitro*. ELAB 60WB adhered to the safety criteria by lacking genes associated with hemolysis and mucin degradation, which may be concerning when administered as a dietary supplement. Whole-genome sequencing revealed a genome size of 1,914,577 bp with a GC content of 37.1%. Phylogenetic analyses revealed close relationships among similar strains. Functional annotation identified 1997 genes, including protein-coding sequences, tRNAs, rRNAs, and tmRNAs, covering 232 SEED subsystems. Within the genome, two bacteriocin genes, penocin A and enterolysin A had the potential to combat the tested pathogens. Manual prediction effectively revealed the presence of probiotic and antibiotic resistance genes in different environments. Carbohydrate metabolism analysis revealed 160 genes in the carbohydrate-active enzymes gene family. Safety assessment revealed no antimicrobial resistance or virulence genes; however, a plasmid, repUS64, was identified, which is common in *Lactobacilli*. Overall, *P. pentosaceus* ELAB 60WB contains carbohydrate metabolism genes and probiotic properties that can lead to its potential use in the food industry and in the treatment of metabolic syndrome.

1. Introduction

Microbiological exploration of probiotics has revealed various microorganisms with potential health benefits applicable to animals and humans. *Pediococcus pentosaceus* is a distinct species among these diverse probiotic strains owing to its genomic composition and probiotic characteristics. Taxonomically, *Pediococcus* belongs to the Lactobacillaceae family and is classified as a lactic acid bacterium (LAB), primarily because of its lactic acid metabolite production in the presence of carbohydrates (Katepogu et al., 2023). Like other members of the LAB, *P. pentosaceus* has gram-positive characteristics, is non-motile, has facultative anaerobic tendencies, and survives in acidic environments, typically between pH 1.0 and 2.0 (S. S. Jiang, Cai, Lv, & Li, 2021). This study explored the probiotic potential and genomic characteristics of *P. pentosaceus* ELAB 60WB, a strain isolated from fermented cherry tomatoes in Lithuania, to reduce the gap between genomic insights and functional probiotic traits.

P. pentosaceus is distinguished by its adaptability across a spectrum of ecological niches, encompassing a diverse array of fermented foods and the intricate microenvironments of the gastrointestinal tract (GIT) found in human and animal ecological niches (Bumbie et al., 2024; Tathode et al., 2024). *P. pentosaceus* is readily cultivated, making it an attractive research area for food industry researchers. As a recognized probiotic, it is extensively used in food products and animal feed. Its health advantages encompass antioxidant properties, the suppression of harmful bacteria, immune system support, obesity prevention, enhancement of the intestinal barrier, and improved gut bacteria balance (S. Jiang et al., 2021; Higashikawa et al., 2016; Shan et al., 2021; Megur et al., 2021). However, notably, apprehensions surrounding consumer safety have increased, particularly in the context of probiotic LAB consumption among immunocompromised individuals (Haranahalli Nataraj et al.,

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Abbreviations:

LAB – Lactic acid bacteria
GIT – Gastrointestinal tract
CDS – Coding DNA Sequence
ANI – Average nucleotide identity
CAZymes – Carbohydrate-active enzymes

2023). Importantly, the functions and safety profiles of probiotics vary among different strains, making broad assumptions impossible. In this context, guaranteeing the safety of probiotic-infused edibles requires a rigorous assessment of safety parameters and potential untoward interactions between probiotics and hosts (S. Jiang et al., 2021). This examination is crucial when considering the frequency of daily consumption of probiotic products.

Probiotics can be evaluated by methodically assigning their functions, which involves identifying the genes necessary for adaptation to different niches, including the gut environment (Peng et al., 2022; Tang et al., 2023). By predicting gene functions, researchers can identify key molecular markers that distinguish potential probiotics from other types of bacteria (Syrokou et al., 2022). *P. pentosaceus* commonly isolated from fermented foods, often possesses genes related to carbohydrate metabolism and bacteriocins, which are crucial for its probiotic potential and hinder pathogens (Tathode et al., 2024). By contrast, less common *P. pentosaceus* strains have been isolated from the GIT of humans, poultry, ducks, and freshwater prawns (Bumbie et al., 2023; Huang et al., 2020). These GIT-isolated strains exhibit unique physiological and metabolic properties compared to foodborne strains (Lu et al., 2024). This is exemplified by the complete genome sequence of approximately 157 *P. pentosaceus* strains, rendering their genetic information publicly accessible. In this study, the complete genome sequencing was followed by a systematic progression of bioinformatics assessments on *P. pentosaceus* ELAB 60WB, isolated fermented cherry tomatoes procured from Vilnius, Lithuania and registered at the National Center for Biotechnology Information (NCBI) submission portal (https://www.ncbi.nlm.nih.gov/nuccore/JAUEMC000000000) (Megur et al., 2023). This study provides insights into genomic characteristics, with a specific emphasis on identifying genes that confer attributes relevant to probiotic potential and safety considerations. We also selected some common foodborne pathogens such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Escherichia coli* as indicator strains because of their considerable impact on food safety (Shi & Kang, 2024). These pathogens are often associated with food poisoning and gastrointestinal infection (Alonso et al., 2019). Their presence in food products pose serious health risk, which is why they are used in probiotic studies to assess the antimicrobial abilities of new probiotic strains. Therefore, evaluating *P. pentosaceus* ELAB 60 WB for its ability to inhibit these pathogens is critical for determining its safety and effectiveness as a probiotic in the food industry.

2. Materials and methods

2.1. Strain isolation

The microbial strain was obtained from fermented cherry tomatoes procured from Hales Turgus Market in Vilnius, Lithuania. The isolation process involved successive dilutions of the samples followed by inoculation on de Man–Rogosa–Sharpe (MRS) agar (Oxoid, Wesel, Germany). Subsequently, the inoculated agar plates were incubated at 37 °C for 48 h, all conducted within an anaerobic environment. Distinct colonies were selected based on their morphological characteristics such as color, shape, and size to ensure that they were representative of the pure culture.

2.2. Tolerance test

MRS broth was adjusted with 1N HCl to pH 2, and 1% prepared bacterial cultures were added to the respective MRS broth in test tubes. NaCl tolerance tests were performed at 6% by increasing the concentration of NaCl in the MRS broth (Prabhurajeshwar & Chandrakanth, 2017). Bile salt tolerance was executed using 0.3% (w/v) bile salt (Sigma-Aldrich, Poznań, Poland) dissolved in MRS broth. The fluid was inoculated with 1% (v/v) bacterial cultures and incubated at 37 °C for 6 h, as previously described (Megur et al., 2023). Gastric juice survival tests were performed using the technique described in the same study. Phenol tolerance tests were performed at 0.1% and 0.4% the increasing concentration of phenol dissolved in the MRS broth (Reuben et al., 2019).

2.3. Antimicrobial activity

The antimicrobial activities of the isolates were tested against the indicator bacteria *S. pyogenes* ATCC 19615, *S. aureus* ATCC 29213, *S. typhimurium* ATCC 14028, *K. pneumoniae* ATCC 13883, and *E. coli* ATCC 25922. The antagonist activity was measured using the standard agar well diffusion technique on Mueller Hinton Agar plates (Sigma-Aldrich, Poznań, Poland). To counteract the inhibitory effects of the metabolite lactic acid, the pH of all supernatants was adjusted to 7.2 using NaOH solution. The zone of inhibition was measured in millimeters after 24 h of incubation at 37 °C (Megur et al., 2023).

2.4. Safety assessment of P. Pentosaceous ELAB 60WB

2.4.1. Hemolytic activity

Hemolytic activity was tested for *P. pentosaceous* ELAB 60WB and the positive control, *S. aureus* ATCC 29213. These isolates were inoculated on a blood agar medium containing 5% sheep blood (Sigma-Aldrich, Poznań, Poland) for 48 h at 37 °C. After incubation, the plates were observed for hemolysis zones around the colonies.

2.4.2. Antibiotic susceptibility test

Antibiotic susceptibility tests were performed as previously described. Briefly, the test has been performed by disc diffusion method against rifampicin, erythromycin, penicillin G, ampicillin, lincomycin, kanamycin, gentamycin, streptomycin, vancomycin, and tetracyclin (Carl Roth, Karlsruhe, Germany) (Megur et al., 2023).

2.4.3. Mucin degradation

The mucin degradation ability of ELAB 60WB was assessed as previously described method (Megur et al., 2023). Briefly, the ELAB 60WB was grown in MRS broth supplemented with 0.5% (w/v) glucose and 0.5% (w/v) mucin from the porcine stomach (Sigma-Aldrich, St. Louis, Missouri, USA). After inoculation, cultures were incubated at 35 °C for 48 h. Bacterial growth was measured every 6 h at an absorbance of 600 nm. The strain *E. coli* ATCC 25922 was used as positive control and grown in Tryptic soy broth (Carl Roth, Karlsruhe, Germany) containing 0.5% (w/v) glucose supplemented with or without 0.5% (w/v) mucin (Sigma-Aldrich, Poznań, Poland) and cultured at 35 °C for 48 h under aerobic conditions. The optical densities were measured using an Eppendorf Biospectrometer (Eppendrof, Hamburg, Germany). The initial optical density of the medium was deduced from the final value of each test sample.

2.5. DNA extraction and whole-genome sequencing

Whole-genome sequencing was performed using a COSMOS ID (Germantown, MD, USA). Briefly, bacterial DNA was isolated using the QIAGEN DNeasy PowerSoil Pro Kit, following the manufacturer's

instructions. The quantity of DNA in the samples was measured using QuantiFluor dsDNA System and GloMax Plate Reader System (Promega Italia, Promega France). DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) and IDT Unique Dual Indexes, starting with 1 ng of DNA input. Genomic DNA was fragmented using the Illumina Nextera XT fragmentation enzyme, and a unique dual index was added. The library was subjected to 12 PCR cycles before being purified with AMpure magnetic beads (Beckman Coulter, Indiana, USA) and eluted with QIAGEN EB buffer. The quantity of DNA in the library was measured using a Qubit 4 fluorometer and a Qubit dsDNA HS Assay Kit. Finally, the library was sequenced on an Illumina NovaSeq platform with 2 × 150 bp reads. The CG view server constructed a circular genomic map from the resultant genome (https://cgview.ca/) (Grant & Stothard, 2008; Megur et al., 2024).

2.6. Genomic comparison

The genome sequence of *P. pentosaceus* ELAB 60WB was compared with that of the strains *P. pentosaceus* DSM20336 (GenBank accession: GCA_001437285.1), *P. pentosaceus* GDIAS001 (GenBank accession: GCA_009791435.1), *P. pentosaceus* ZZ61 (GenBank accession: GCA_030480445.1), *P. pentosaceus* MR001 (GenBank accession: GCA_019614475.1), *P. pentosaceus* SM2D17 (GenBank accession: GCA_035209715.1), and *P. pentosaceus* LA0061 (GeneBank accession: GCA_033882205.1) using Basic Local Alignment Search Tool (BLAST)+ 2.12.0 (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and FastANI software for detecting structural differences, thereby enabling a comparative genome analysis to elucidate the relationship between mutations and strain evolution.

2.7. Phylogenetic analysis

To identify taxonomy, average nucleotide identity (ANI) based on the BLASTN algorithm (ANIb), ANI based on the MUMMER ultrafast alignment tool (ANIm), tetranucleotide frequency correlation coefficients (TETRA), and indices of the *P. pentosaceus* ELAB 60WB strain were calculated using the JSpecies Web Server (Richter et al., 2016). The Type Genome Server (TYGS) (https://tygs.dsmz.de/) (Meier-Kolthoff & Göker, 2019) was also used to create a bootstrapped phylogenetic relationship through pairwise comparison of genome sequences.

2.8. Functional annotation and gene prediction

Genome annotation was performed using the Prokaryotic Genome Annotation System (Prokka) (Seemann, 2014) and Rapid Annotations using the Subsystems Technology (RAST) webserver (https://rast.nmpdr.org/) (Meier-Kolthoff & Göker, 2019). In addition, the functional annotations were carried out using the BLASTKOALA (https://www.kegg.jp/blastkoala/) (Kanehisa et al., 2016) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/pathway.html). The most important genes related to probiotic properties were manually selected from the Prokka, RAST, and KEGG-derived annotations (Megur et al., 2024).

2.9. Bacteriocin identification

The strain was assessed for the presence of bacteriocin operons by BAGEL4 (http://bagel4.molgenrug.nl/) and the domains of bacteriocin were determined using BLASTP analysis against the non-redundant protein databases created by BLASTP from the NCBI.

2.10. Carbohydrate metabolism

The annotated genomes of the ELAB 60WB were searched against the CAZY database (http://www.cazy.org/) on dbCAN2 (http://bcb.unl.edu/dbCAN2/index.php), an online webserver, using DIAMOND BLAST search with parameter E-Value < 1e−102 and ≥80% identity.

2.11. Genomic safety evaluation

To identify antimicrobial resistance genes in the ELAB 60WB genome, we used the ResFinder tool v.4.3.3 from the Center for Genomic Epidemiology. For virulence determinants, we consulted Virulence Finder v.2.0.3 and the Virulence Finder and the Virulence Factor of Bacterial Pathogen Database (Liu et al., 2022). Plasmids were detected using the PlasmidFinder tool of the Center for Genomic Epidemiology (https://cge.food.dtu.dk/services/PlasmidFinder/).

2.12. Statistical analysis

All experiments were performed in triplicate. Data were analyzed using GraphPad Prism version 8.4.3 (GraphPad Software Inc., Boston, United States). A one-way analysis of variance(ANOVA) was used to analyze the data, and Tukey's test was performed to determine significant differences ($p < 0.05$) between the effects expressed as mean ± standard deviation calculated at a 95% confidence interval (CI). Tukey's test was used to examine the differences between the means, and statistical significance was set at $p < 0.05$.

3. Results and discussion

In this study, we isolated the *P. pentosaceus* ELAB 60WB strain from fermented cherry tomatoes procured from the Hales Turgus Market, Vilnius, Lithuania (Megur et al., 2023). Isolation of *P. pentosaceus* strains from fermented foods has been extensively studied because of their potential as probiotics and their role in food preservation. Various *P. pentosaceus* strains have been isolated from different fermented foods, including marula, fermented sausages, kimchi, and traditional fermented beverages such as boza (Rodrigues Blanco et al., 2022; Tathode et al., 2024)(J. Jiang et al., 2020). These findings highlight the importance of *P. pentosaceus* in fermented foods for their role in food preservation and their potential to survive in the GIT and produce metabolites for health improvement.

3.1. Tolerance test

P. pentosaceus ELAB 60WB grew (optical density [OD] = 0.650) in 6% NaCl concentration compared to blank (OD = 0.010). In this *in vitro* assessment of the study, the strain exhibited the capacity to withstand and grow within 6% NaCl-containing broth. This is an important feature of probiotic strains that can tolerate a wide range of salt concentrations (Khushboo et al., 2023). Similarly, the study on the *P. pentosaceus* MR001 indicated tolerance up to 6% NaCl in MRS broth, maintaining a viable count of $7.86 \pm 0.09 \times 10^{10}$ CFU mL (Wanna et al., 2021). In 0.4% phenol solution, the indicator of growth, in terms of OD, was 0.545, representing the degree of tolerance. For resistance to 0.3% bile salt concentration, the strain showed growth on the MRS agar plates after overnight incubation (Table 1). Phenol tolerance and liver bile salt tolerance are key features of a probiotic strain that allow it to survive and thrive in the GIT (Battistini et al., 2023; Reuben et al., 2019). Gastric juice (pH 2.0) is bactericidal to most microbes, except enteric bacteria, when ingested in the GIT. *P. pentosaceus* ELAB 60WB strain showed the capacity to survive and grow within low pH adjusted MRS broth after 4 h of incubation. These *in vitro* physiochemical tests showed that *P. pentosaceus* ELAB 60WB can survive in the competitive environment of the GIT.

3.2. Antimicrobial activity

Antimicrobial activity is defined as the antagonism between different microbial organisms for food sources and territory, and it is a common biological function exhibited by different groups of bacteria to either kill

Table 1
In vitro probiotic properties of the *Pediococcus pentosaceus* ELAB 60WB isolate. (+) Positive growth. (−) No effect. RIF – rifampicin, E – erythromycin, P – penicillin G, AMP – ampicillin, L – lincomycin, K – kanamycin, GEN – gentamycin, S – streptomycin, VA – vancomycin, and TE – tetracyclin.

In vitro tests	Results
6% NaCl tolerance test (OD_{600nm})	+ (0.650)
0.3% Bile salt tolerance test	+ (55%)
0.4% Phenol tolerance test (OD_{620nm})	+ (0.545)
Antagonistic ability against *Streptococcus pyogenes* ATCC 19615 (zone diameter in mm)	21
Antagonistic ability against *Staphylococcus aureus* ATCC 29213 (zone diameter in mm)	14
Antagonistic ability against *Salmonella typhimurium* ATCC 14028 (zone diameter in mm)	26
Antagonistic ability against *Klebsiella pneumoniae* ATCC 13883 (zone diameter in mm)	16
Antagonistic ability against *Escherichia coli* ATCC 25922 (zone diameter in mm)	–
Antibiotic resistance	TE, P, E, VA, and L
Antibiotic susceptibility	RIF, AMP, GEN, and S
Gastric juice survivability (pH 2.0)	+ (45%)
Ability to grow at 48 °C	+

or inhibit the growth of microorganisms (Bodke & Jogdand, 2022). However, the degree of antagonism varies depending on the particular strain and can exert its effects through various mechanisms, including the disruption of cell wall synthesis, inhibition of protein synthesis, and interference with nucleic acid metabolism (Yang et al., 2022). Regarding the antagonistic ability of *P. pentosaceus* ELAB 60WB, the diameter of the zone of inhibition ranged 14–25 mm for the five different infectious bacterial strains. The antagonistic ability against *S. pyogenes* ATCC 19615 was 21 mm, against *S. aureus* ATCC 29213 was 14 mm, against *S. typhimurium* ATCC 14028 was 26 mm, against *K. pneumoniae* ATCC 13883 was 16 mm, and no antimicrobial effect was observed against *E. coli* ATCC 25922 (Table 1, Supplementary Fig. 1). Different strains of *P. pentosaceus* produce bacteriocins, bacteriocin-like inhibitory substances, and metabolites such as hydrogen peroxide and diacetyl, which contribute to their overall antimicrobial properties (de Azevedo et al., 2019)(S. K. S. K. Park, Jin, Song, & Baik, 2023). *P. pentosaceus* produces ribosomally synthesized antimicrobial peptides, such as pediocins, belonging to Class IIa, which are known for their broad-spectrum activity against gram-positive food-borne pathogens such as *Listeria monocytogenes* (S. K. Park, Song, et al., 2023). The antimicrobial activity of *P. pentosaceus* BLIS demonstrated its potential as a natural antimicrobial agent against *S. aureus in vitro*, demonstrating its potential for application in the food industry (de Azevedo et al., 2019).

3.3. Safety assessment

3.3.1. Hemolytic activity

The hemolytic activity of bacteria is the ability to break down blood cells, also known as β-hemolytic effect. *P. pentosaceus* ELAB 60WB exhibited a non-hemolytic character, classified as γ hemolysis, after inoculation on blood agar plates. Contrastingly, a distinct transparent circle was observed around *S. aureus* ATCC 29213, which served as the control (Fig. 1A). These results were consistent across several *P. pentosaceus* strains, suggesting the absence of hemolytic activity, a common characteristic of this species (S. Park, Song, et al., 2023; Zommiti et al., 2018). Hemolysis is an important virulence factor, and the lack of hemolytic activity supports the safety of *P. pentosaceus* for use in food applications and as a potential probiotic (Zaghloul & Halfawy, 2024).

3.3.2. Antibiotic activity

Therefore, the susceptibility of all potential probiotic strains to an array of commonly used antibiotics should be assessed. This procedure is essential for the detection of potential probiotic strains with transferable antibiotic resistance plasmids. Antibiotic susceptibility testing revealed that the isolate was sensitive to rifampicin, ampicillin, gentamicin, and streptomycin. This strain was resistant to erythromycin, penicillin G, lincomycin, kanamycin, vancomycin, and tetracycline (Table 1). The resistance of *P. pentosaceus* to different antibiotics is due to the presence of resistance genes, such as *ermB* and *msrC*, which confer resistance to specific antibiotics within the macrolide-lincosamide-streptogramin B group. (Thumu & Halami, 2019). By contrast, *P. pentosaceus* commonly found in fermented foods, has adapted to survive under various stressful conditions, including antibiotics. This adaptability may have contributed to its ability to develop resistance over time, especially when introduced into antibiotics used in food production and treatment (Lin et al., 2019; Raccach, 2014). Horizontal gene transfer (HGT) is very common in *P. pentosaceus* strains, where the transfer of antibiotic resistance genes to other bacteria occurs via conjugation. Plasmids containing resistance genes are shared with pathogenic bacteria, complicating treatment options for infections (J. Jiang et al., 2020).

3.3.3. Mucin degradation

P. pentosaceus ELAB 60WB did not degrade mucin compared to the positive control *E. coli* 35150 (Fig. 1B). Mucin is a glycoprotein that forms the mucus layer in GIT and provides a physical barrier against pathogens (Yamaguchi & Yamamoto, 2023). Certain pathogenic bacteria produce specific enzymes such as glycoside hydrolases, which can break down glycosidic bonds in mucin (Glover et al., 2022). The degradation of the mucus layer by non-beneficial bacteria in the colon increases permeability and inflammation and alters the microbiota composition (Schroeder, 2019). Research has indicated that certain strains of *P. pentosaceus* do not possess mucin degradation capabilities, suggesting a lack of ability to use mucin as a carbon source, which has demonstrated safety in animal studies (S. Jiang et al., 2021).

3.4. Genome characteristics of P. pentosaceus ELAB 60WB

The complete *P. pentosaceus* ELAB 60WB genome contained a single circular chromosome of 1,914,577 bp with a guanine–cytosine (GC) ratio of 37.1% (Fig. 2, Table 2). Whole-genome sequences of *P. pentosaceus* ELAB 60WB were submitted to the NCBI for Biotechnology Information submission portal (https://www.ncbi.nlm.nih.gov/nuccore/JAUEMC000000000). The genome size was smaller than that of *P. pentosaceus* ST65ACC (1,933,194 bp) (Oliveira, da Silva Rodrigues, de Carvalho, & Nero, 2023) and larger than that of *P. pentosaceus* GDIAS 001 (1,831,351 bp) (Song et al., 2020) and *P. pentosaceus* LI05 (1,751,578 bp) (Lv et al., 2014). Previous research on the complete genome sequencing of *P. pentosaceus* indicates that the genome size for this strain ranges 1.7–2.11 Mb. These studies established a link between the number of genes and their prevalence, concluding that microbes with large genomes exhibit superior adaptation to diverse environments (J. Jiang et al., 2020; Zaghloul & Halfawy, 2024; Nikodinoska et al., 2022). The average GC content was 37.26%, with variations observed among strains ranging 35.11%–37.03% in *P. pentosaceus* strains (J. Jiang et al., 2020). The average number of predicted coding DNA sequence (CDS) per genome ranged 1692–2170, whereas ELAB 60WB had a CDS count of 1941. These characteristics highlight the genetic diversity and functional capabilities of *P. pentosaceus*, which make it a versatile species.

3.5. Genome comparison analysis of P. pentosaceus ELAB 60WB and the other six P. pentosaceus strains

Comparative genomic analysis of different *P. pentosaceus* genomes has provided insights into the genetic diversity, genomic structure, and potential biotechnological applications of this species. The comparison of the genomes of six different closely related *P. pentosaceus* strains with

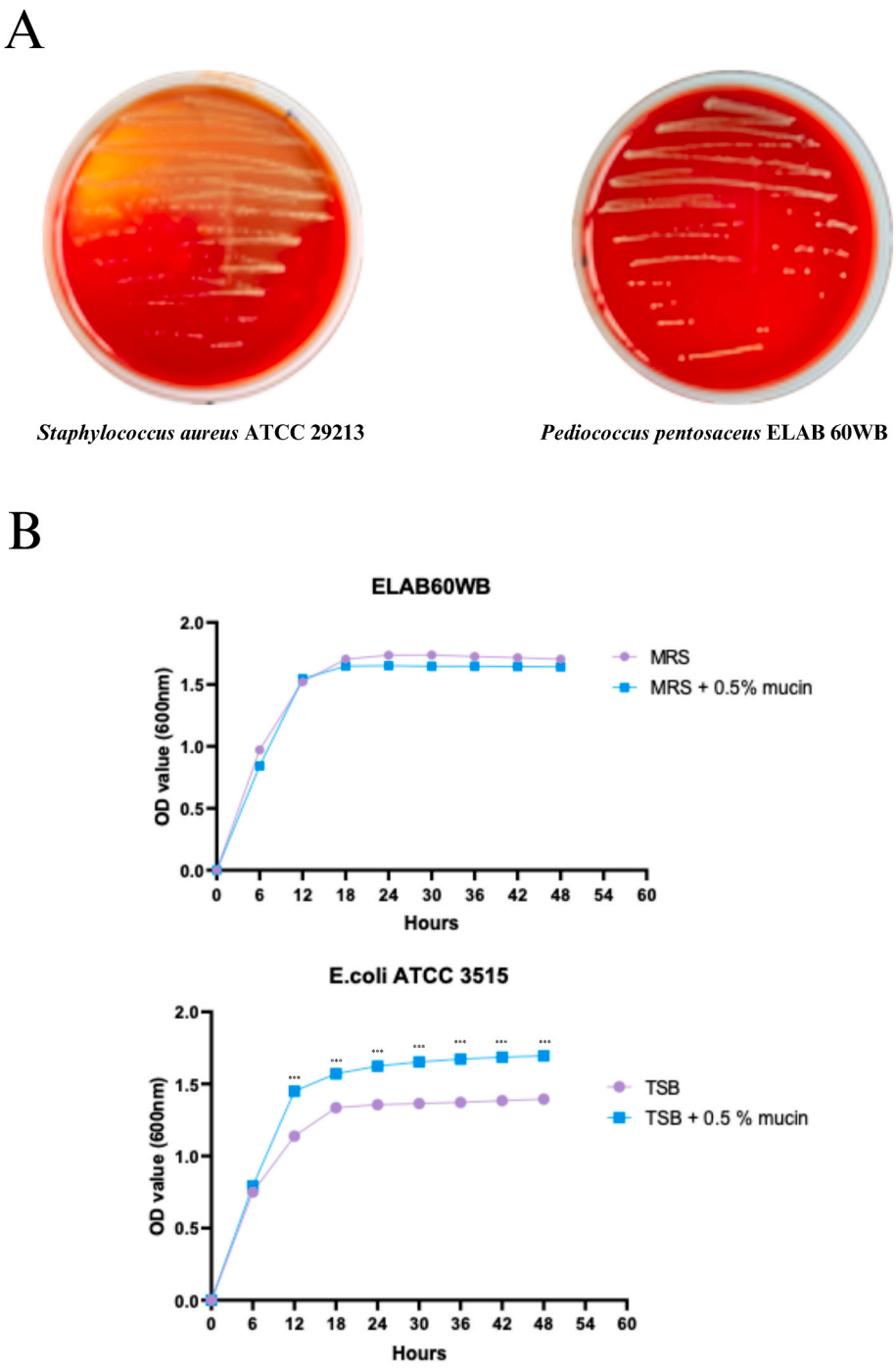

Fig. 1. Safety evaluation test of *Pediococus pentosaceus* ELAB 60WB. **A)** Assessment of hemolytic activity of positive control *Staphylococcus aureus* ATCC 29213 and *P. pentosaceus* ELAB 60WB. **B)** Mucin degradation ability of *P. pentosaceus* ELAB 60WB and *Escherechia coli* ATCC 3515, a positive control. ***significant difference at $p < 0.001$.

the genome of ELAB 60WB revealed structural differences in the prophage regions, combatting stress responses, secondary metabolite biosynthesis, and HGT regions (Fig. 3). The genomic diversity within *P. pentosaceus* strains is significant, with variations observed primarily in carbohydrate metabolism and horizontally transferred DNA, such as prophages and bacteriocins encoded on plasmids (J. Jiang et al., 2020). Prophage regions are common in *P. pentosaceus* genomes, contributing to genetic diversity and potentially influencing the behavior and survival (J. Jiang et al., 2020).

FastANI supported the visualization of orthologous mapping and similarities among the six different strains of *P. pentosaceus*. ANI was computed using *P. pentosaceus* ELAB 60WB as the reference strain and *P. pentosaceus* DSM20336, *P. pentosaceus* GDIAS001, *P. pentosaceus* ZZ61, *P. pentosaceus* MR001, *P. pentosaceus* SM2D17, and *P. pentosaceus* LA0061 as the query strains (Fig. 4). This revealed genetic similarity between the query and reference genomes. FastANI has been used in numerous studies to verify the identity of probiotic strains by comparing their genomes with those of reference strains. In a study on probiotic strains, FastANI assisted with the safety assessment of probiotic strains, which involved identifying potentially harmful genomic features and comparing them across different strains to ensure probiotic safety for consumption (Umanets et al., 2023). FastANI also enables large-scale

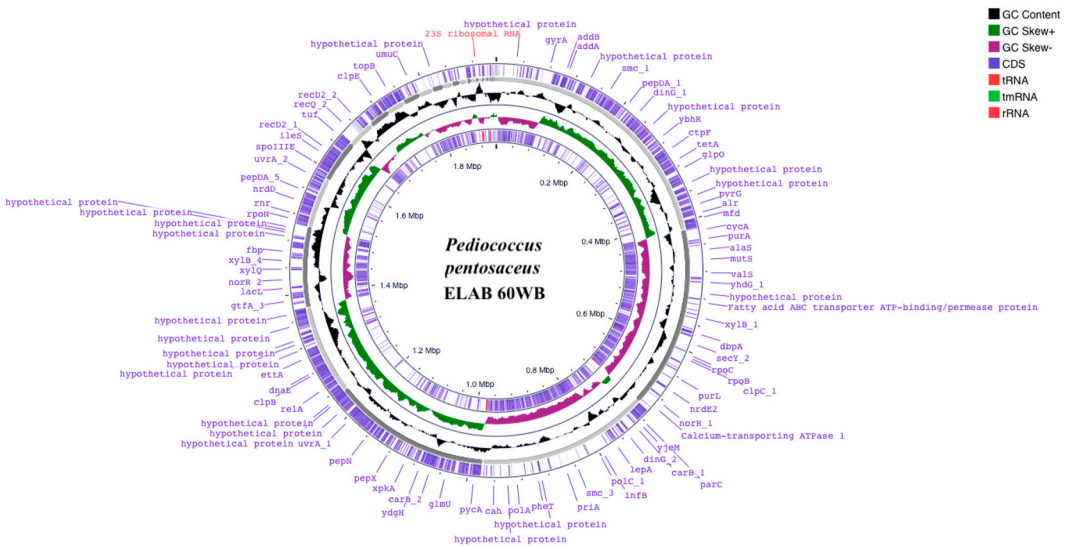

Fig. 2. Circular genome map of *P. pentosaceus* ELAB 60WB. The circular illustration was constructed using the CGView tool. Genomic features are represented from the outer to the inner circle, Circle 1 illustrates Prokka annotated forward and coding DNA sequences (CDS) with tRNA, rRNA, and tmRNA; circle 2 represents the guanine–cytosine (GC) content; circle 3 displays the GC skew (G–C)/(G + C); and circle 4 shows the genome size, 1,914,577 bp.

Table 2
General genomic features of *P. pentosaceus* ELAB 60WB.

Attribute	Value
Genome size	1,914,577 bp
GC content	37.1%
Number of contigs	33
N50 (bp)	274,435
L50	3
CDS	1941
Number of RNAs	54 (50 tRNA + 3 rRNA + 1 tmRNA)
Total genes	1997
Protein coding genes	1943
Genes assigned to COGs	807
Accession no.	JAUEMC000000000

genome analysis, which can be used to compare LAB strains from the gut and fermented food environments, thus revealing the genetic relationships and potential origins of probiotic bacteria (Pasolli et al., 2020).

3.6. Phylogenetic analysis

The similarity of *P. pentosaceus* ELAB 60WB was compared with other genomes of *P. pentosaceus* deposited in the NCBI database using ANIb, ANIm, and TETRA. The closest values of strain ELAB 60WB to all evaluated *P. pentosaceus* strains were above the limit established for each algorithm (ANIb, 95%; ANIm, 95%; and TETRA, 0.99). *P. pentosaceus* ELAB 60WB was identified as the closest neighbor of the strain, with a genome similarity of 98.48% for ANIb, 98.81% for ANIm, and 0.9983% for TETRA. High similarity between the genomes of *P. pentosaceus* was also observed in the phylogenomic tree inferred from the TYGS-genome BLAST distance phylogeny distances in TYGS (Fig. 5). These results indicated that the ELAB 60WB strain unequivocally belonged to *P. pentosaceus*. A previous study of 74 *P. pentosaceus* genomes revealed six major branches. This analysis showed that the strains were not correlated based on the isolation source or sampling region (J. Jiang et al., 2020). Different strains of *P. pentosaceus* produce bacteriocins that differ in structure and are crucial for preventing pathogenic bacterial growth in the gut and food fermentation (Rodrigues Blanco et al., 2022). In summary, *P. pentosaceus* strains exhibit complex and diverse genomic structures, particularly in their ability to produce bacteriocins and their resistance mechanisms.

3.7. Functional annotation and gene prediction

Genome annotation using Prokka predicted 1997 genes, including 1943 CDS, 50 tRNAs, 3 rRNAs, and 1 tmRNA (Table 2). Of the predicted CDS, 1189 genes (61.20%) were functional and 754 genes (38.80%) were hypothetical/unknown. The 50 tRNA sequences correspond to 21 natural amino acids: Leu (five sequences), Arg (five sequences), Asn (two sequences), Gly (four sequences), Lys (two sequences), Met (four sequences), Ser (four sequences), Thr (four sequences), Asp (three sequences), Gln (two sequences), Glu (three sequences), Pro (two sequences), Phe (two sequences) Val (three sequences), His (one sequence), Tyr (one sequence), Ala (one sequence), Cys (one sequence), and Ile (one sequence).

Analysis of the genome on the RAST webserver provided an overview of the coded biological features N50 (bp) 274,435 with a subsystem coverage of 29%, distributed across 232 SEED subsystems (Fig. 6). The distribution of different functional groups revealed a predominance of genes involved in general processes related to protein, carbohydrate, amino acid, and amino acid derivative metabolism. A total of 53 genes were involved in the synthesis of cofactors, vitamins, prosthetic groups, and pigments, leading to the biosynthesis of biotin, riboflavin, thiamine, pyridoxine, folate, and lipoic acid. These biosynthesized byproducts play critical roles in various biological processes, including cellular signalling, energy metabolism, and interactions with the host, leading to the synthesis and transport of vitamin B (Lv et al., 2014; Tathode et al., 2024), displaying ELAB 60WB as a desirable trait of a probiotic strain. In the carbohydrate category, 103 genes were identified, 13 of which were involved in the fermentation process (eight genes for lactate fermentation and five genes for acetoin and butanediol metabolism). The fermentation genes in *P. pentosaceus* strains are primarily involved in carbohydrate metabolism, including glycolysis and use of various sugars (J. Jiang et al., 2020). Genes related to the synthesis of vitamin B and carbohydrate metabolism make strain ELAB 60WB essential for biotechnological and probiotic applications in fermented foods and preservation.

Clusters of Orthologous Groups (COG) analysis using the EggNOG mapper v2 assigned 1799 genes into 19 clusters (Fig. 4). Higher numbers were sorted under function unknown (S: 356), which revealed the unpredicted potential genes of this strain. The remaining proteins were categorized under functional groups such as nucleotide transport and metabolism (F: 98); carbohydrate transport and metabolism (G: 167);

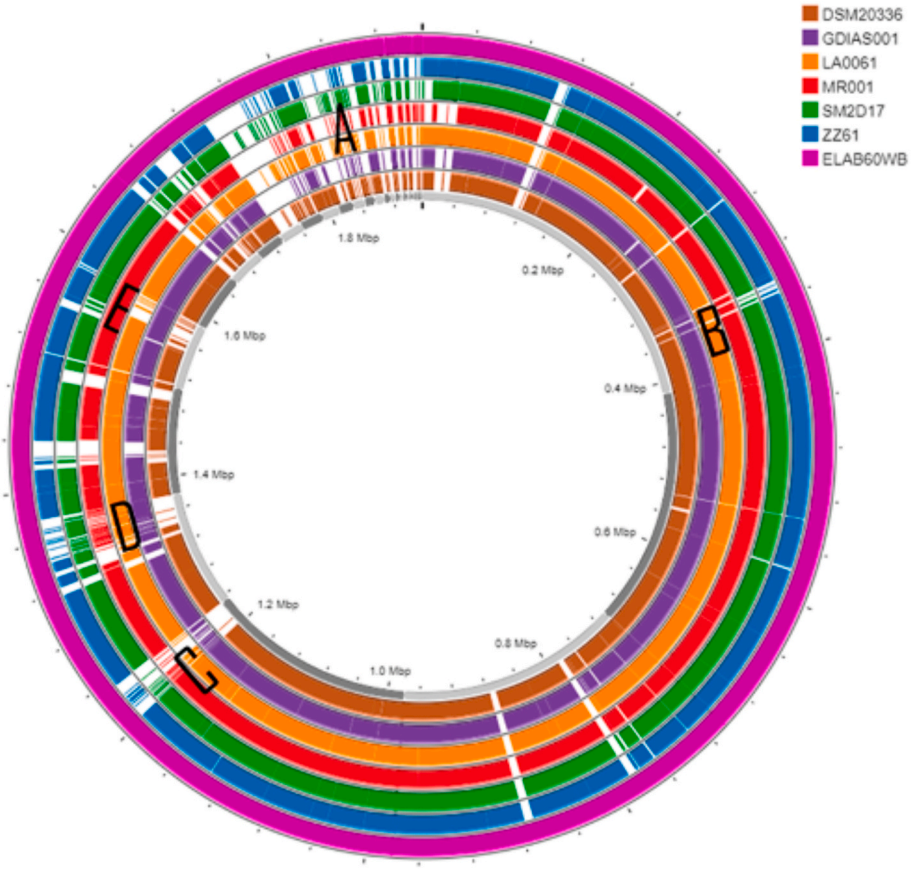

Fig. 3. Genome comparison using bidirectional BLASTP of *P. pentosaceus* species with the circular genome viewer Proksee. Genomic features are represented from the outer to the inner circle. Circle 1 shows the genome content of ELAB 60WB. Circle 2 (ZZ61), circle 3 (SM2D17), circle 4 (MR001), circle 5 (LA0061), circle 6 (GDIAS001), and circle 7 (DSM20336) illustrates the genomic variations between the different *p. pentosaceus* strain with ELAB 60WB genome. Region A primarily includes the genes related to amino acids, defence mechanisms, metabolism, and carbohydrate transfer. Region B primarily includes cell wall, membrane, and envelope biogenesis. Regions C and D are phage regions. Region E comprises of genes related to stress response and secondary metabolites biosynthesis.

transcription (K: 168); energy production and conversion (C: 64); amino acid transport and metabolism (E: 122); replication, recombination, and repair (L: 152); translation, ribosomal structure, and biogenesis (J: 161); cell wall/membrane/envelope biogenesis (M: 111); inorganic ion transport and metabolism (P: 83); coenzyme transport and metabolism (H: 48); intracellular trafficking, secretion, and vesicular transport (U: 54); signal transduction mechanisms (T: 43); defence mechanisms (V: 46); lipid transport and metabolism (I: 46); posttranslational modification, protein turnover, and chaperones (O: 39); cell cycle control, cell division, and chromosome partitioning (D: 29); secondary metabolites biosynthesis, transport, and catabolism (Q: 15); and cell motility (N: 8) (Fig. 7).

The KEGG functional annotation using BLASTKOALA assigned approximately half of the genes (52.4%, 1046 genes) into 22 different functional categories (Table 3), mostly related to protein families: genetic information processing (317 genes), carbohydrate metabolism (130 genes), protein families: signalling and cellular processes (115 genes), environmental information processing (64 genes), genetic information processing (22 genes), lipid metabolism (33 genes), amino acid metabolism (33 genes), nucleotide metabolism (57 genes), and human diseases (2 genes) (Table 3). The core genome of *P. pentosaceus* strains includes genes related to essential functions such as ribosomal structure, biogenesis, translation, and signal transduction. These core genes are conserved across various strains, and are critical for basic cellular processes (J. Jiang et al., 2020).

Functional annotations by both COG and KEGG searches indicated that ELAB 60WB had a high number of genes associated with genetic information and processing, signaling, and cellular processes, followed by carbohydrate metabolism, suggesting their importance in the conserved cellular processes of our strain to take advantage of a large variety of niches. *P. pentosaceus* encodes proteins related to essential cellular processes such as ribosomal structure and biogenesis, translation, and signal transduction mechanisms (J. Jiang et al., 2020). The genomes of *P. pentosaceus* strains contain genes responsible for carbohydrate metabolism involved in central carbohydrate metabolism, degradation of different compounds, transport mechanisms, and biosynthesis (Tathode et al., 2024). These functionally annotated genes highlight the complex and diverse nature of *P. pentosaceus* genome, which has adapted to various ecological niches and has acquired a range of functional genes. The identified functional genes suggested that *P. pentosaceus* ELAB 60WB can be used to formulate functional foods aimed at enhancing gut health.

3.8. Probiotic properties

An in-depth study of the whole-genome sequence of this strain identified unique *Pediococcus* probiotic genes. Using the existing information available in the literature, manual analysis, and prediction were performed on the genome of *P. pentosaceus* ELAB 60WB. The objective of this study was to identify genetic constituents linked to diverse probiotic features, including stress tolerance, bile salt hydrolase functionality, adhesive capacity, and immunomodulatory properties, thereby elucidating genomic probiotic efficacy. In our genomic analysis, a repertoire of genes encoding proteins associated with stress response was ascertained, as shown in Table 4.

Nine heat shock protein-related genes were identified within the

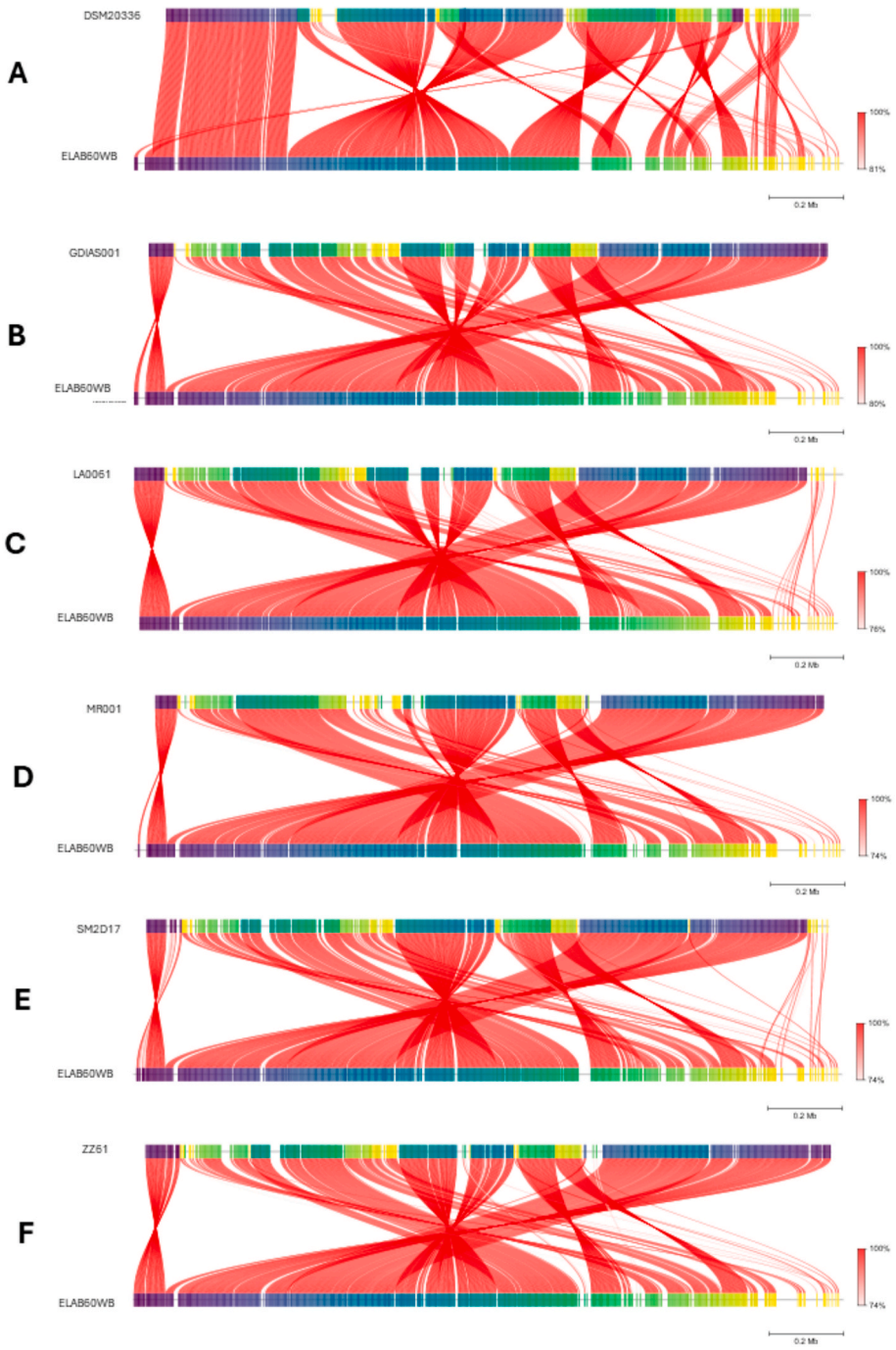

Fig. 4. Comparative analysis of *P. pentosaceus* ELAB 60WB and six other *P. pentosaceus* strains using FastANI. The ANI was computed between query strains a) DSM20336, b) GDIAS001, c) LA0061, d) MR001, e) SM2D17, and f) ZZ61, with the reference strain ELAB 60WB. The red line segments denote the orthologous mappings computed by FastANI for ANI estimation.

genomic composition of ELAB 60WB. These genes have prominent functions in managing intracellular protein aggregation and stabilising membrane integrity, thereby conferring heightened heat resistance within *Pediococcus* strains (Asami et al., 2017; Baig et al., 2021). Conversely, ELAB 60WB has a pair of cold shock protein genes, *csp*C and *csp*LA, which facilitate survival at lower temperatures (Baig et al., 2021; Zhou et al., 2021). Cold-shock proteins are produced in various LABs as countermeasures against the adverse effects of cold stress (Duru et al., 2021; Wouters et al., 1999). Therefore, it is possible that ELAB 60WB serves a comparable function.

The genome of ELAB 60WB encompasses five genes responsible for conferring resistance under low-pH conditions. These genes encode the F_0F_1-ATP synthase subunit A–H cluster, which is recognized as a pivotal modulator of cytoplasmic pH and promotes acid tolerance (Yan et al., 2022). The genes *gad*C and *dna*K, identified in ELAB 60WB, are indispensable for viability in acidic environments. This observation aligns with prior investigations that focused on the viable count of *Lactobacilli* during fermentation processes (L. L. Gong, Ren, & Xu, 2019; Daliri, 2023). Furthermore, our investigation revealed the presence of *nha*C (Makete & Mamphogoro, 2023) and *nha*K (Kiousi et al., 2023) genes encoding sodium-proton (Na^+/H^+) antiporters responsible for maintaining the pH and sodium ion equilibrium, an alkaline shock protein

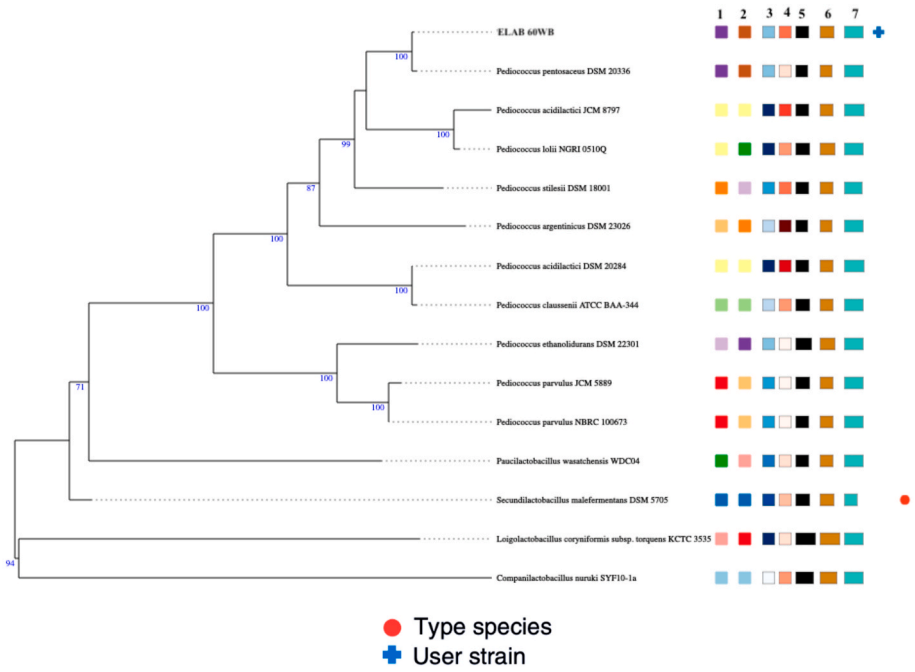

Fig. 5. Phylogenetic comparison of *P. pentosaceus* ELAB 60WB with representative complete genomes of other *Pediococcus* strains performed using the TYGS webserver. Leaf labels were annotated by affiliation to species (1), and subspecies (2) clusters, genomic G + C content (3), δ values (4), overall genome sequence length (5), number of proteins (6), and the type of strain (7). The resulting intergenomic distances were used to infer a balanced minimum evolution tree with branch support using FASTME 2.1.6.1 and calculated using 16S rDNA gene sequencing. The confidence degree of each branch represented before each node is the bootstrap support value.

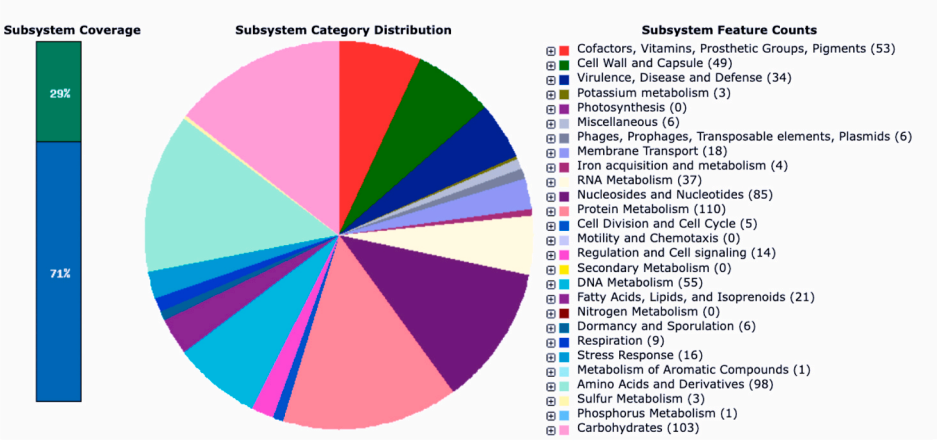

Fig. 6. Overview of the biological subsystem distribution of the genes of *P. pentosaceus* ELAB 60 WB using RAST annotation.

gene denoted as Asp23, and the *gad*C gene encoding glutamate decarboxylase, as reported in a previous study (Phuengjayaem et al., 2023). The *gad*C facilitates the translocation of intracellular gamma-aminobutyric acid (GABA) through extracellular glutamate exchange, thereby permitting the efflux of GABA from the intracellular to the extracellular environment. As a result, *gad* genes can help certain microbial strains produce increased levels of GABA under difficult environmental conditions and are also linked to probiotic qualities (Phuengjayaem et al., 2023).

Probiotics possess antimicrobial properties that assist in regulating the gut environment by promoting the growth of beneficial bacteria. Certain genes encode proteins that inhibit the proliferation of harmful bacteria (Jia et al., 2017). The ELAB 60WB strain contains the *lux*S gene, which enables autoinduction and facilitates communication among bacteria to coordinate responses to changes in population density. This collective behavior allows bacteria to adapt effectively and react to environmental stimuli (Xiao et al., 2023).

Regarding bile salt resistance, the presence of specific genes was observed: *ppa*C, which encodes an inorganic pyrophosphatase that contributes to surface tension maintenance and membrane integrity, and *cfa*, which encodes cyclopropane-fatty-acyl-phospholipid synthase, thereby augmenting lipid synthesis. Analogous mechanisms of bile resistance have been identified in *Lactococcus petauri* LZys1 through combined analysis of its genome and phenotypic traits (Li et al., 2021).

The ability of probiotic strains to adhere to the host epithelial lining is attributed to the presence of specific cell surface proteins. Within the ELAB 60WB genome, *fbp* was likely responsible for encoding proteins associated with adhesion. Similar adhesion-associated genes have been previously documented in strains of *Lactiplantibacillus plantarum* that exhibit notable adhesion proficiency (Sun et al.,

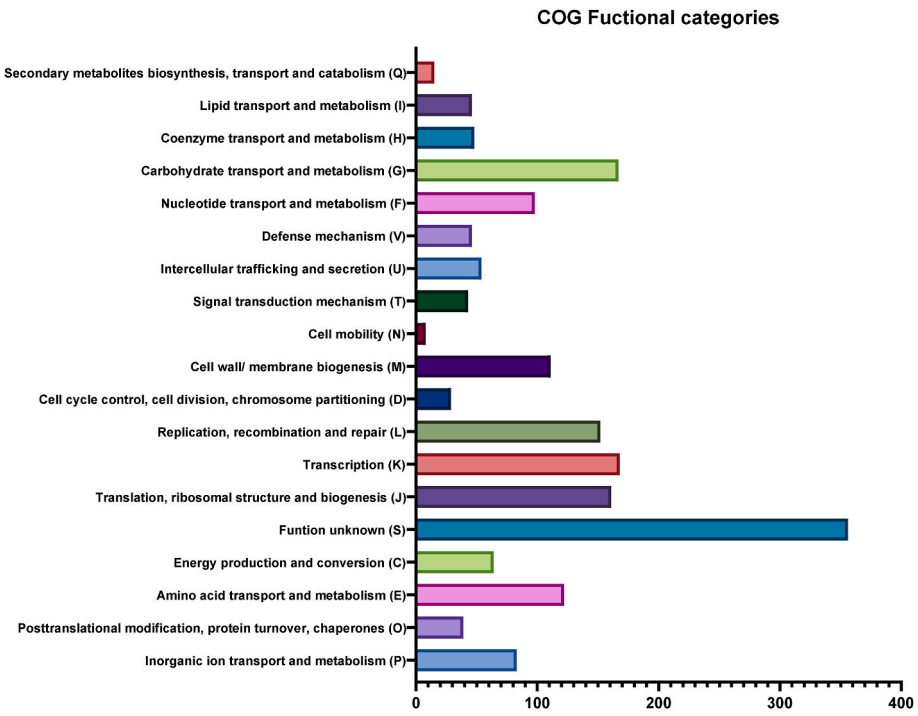

Fig. 7. Visual distribution of clusters of orthologous groups (COG) functional categories of *P. pentosaceus* ELAB 60WB proteins. Y-axis, F: Nucleotide transport and metabolism; G: carbohydrate transport metabolism; H: coenzyme transport and metabolism; I: lipid transport and metabolism; J: translation, ribosomal structure, and biogenesis; L: replication, recombination, and repair; M: cell wall, membrane, and envelope biogenesis; N: cell motility; O: Post-translational modifications, protein turnover, and chaperones; Q: Secondary metabolites biosynthesis, transport, and catabolism, U: Intracellular trafficking, secretion, and vesicular transport; S: Function unknown, E: Amino acid transport and metabolism; D: Cell cycle control, cell division, and chromosome partitioning; C: Energy production and conversion; V: Defense mechanisms; T: Signal transduction mechanisms; K: Transcription; and P: Inorganic ion transport and metabolism.

2022). The *pde*K gene *Pediococcus* species encodes for putative adhesion protein that plays a crucial role in attachment to intestinal epithelial cells (J. Jiang et al., 2020). In probiotic bacteria, enolase is present on the cell surface and plays a role in adhesion to host tissues. Comparative genome analysis of *P. pentosaceus* strains revealed similarities in adhesion-related genes between marine isolates and the probiotic strain MR001 (Zaghloul & Halfawy, 2024).

Moreover, the genetic loci containing *dlt*C and *dlt*D, which are responsible for immunomodulatory functions, were identified in the ELAB 60WB genome. Similar findings have been reported for other LAB strains with genes associated with immunomodulation (Albarracin et al., 2022; Lebeer et al., 2008).

Probiotic strains may possess genes responsible for multidrug resistance, allowing them to survive in the presence of different antibiotics. In ELAB 60WB, eight genes encoding antibiotic resistance proteins were identified, including *emr*B, *mep*A, *mdt*L, and *lmr*A, which belong to the family of multidrug efflux pumps in bacteria (Tóth et al., 2021). These pumps help bacteria to resist antimicrobial compounds by actively removing them from the cell and lowering their internal concentrations. This trait is crucial for the probiotic strains to thrive in the presence of antibiotics (Blair et al., 2014). The *tet*A gene, responsible for tetracycline resistance, was discovered in the ELAB 60WB genome as a response to combat the effects of tetracycline antibiotics by actively pumping antibiotics from the cell. Similar research revealed the presence of the *tet*A gene in probiotics, allowing them to survive in the presence of tetracycline (Mawardi et al., 2023).

ELAB60 WB contains the *trp*S2 gene, which encodes tryptophan synthase beta chain (*Trp*B) in bacteria. *Trp*B is a component of tryptophan synthase, which is crucial for synthesizing the amino acid tryptophan (Han et al., 2020). Particularly, *Trp*B aids the combination of indole and serine to form tryptophan (Wang et al., 2022). This essential step in tryptophan production is vital for protein synthesis and other cellular processes that rely on tryptophan (Wang et al., 2022).

These findings suggest that *P. pentosaceus* ELAB 60WB is resistant to various stressors, allowing it to thrive under diverse GIT conditions. Moreover, its adhesive proteins aid in gut colonization and exert antimicrobial effects against pathogenic microorganisms. In addition, the presence of antibiotic resistance genes enables the bacterium to withstand a variety of antibiotics. Finally, this bacterium produces tryptophan, an amino acid essential for the synthesis of serotonin and melatonin. Comparable outcomes were also noted in earlier physicochemical assessments.

3.9. Bacteriocin

The BLAST results of the BAGEL5 web server for *P. pentosaceus* ELAB 60WB genome predicted two bacteriocin clusters as areas of interest: (i) Penocin A contig 1 (start at 1 and end at 14152) and (ii) enterolysin at contig 4 (start at 239429 and end at 253951) (Fig. 8). Penocin A and enterolysin A bacteriocins were also identified in the genome. A novel pediocin-like bacteriocin, termed penocin A, identified a new class IIa bacteriocin in the genome of *P. pentosaceus* ATCC25745 using silico-based analysis (Diep et al., 2006). Penocin A exhibits a broad antimicrobial spectrum that hinders the proliferation of the pathogenic bacteria, *L. monocytogenes* and *S. aureus*, which are important for maintaining the quality and safety of fermented foods (Diep et al., 2006; Hartmann et al., 2011; Kuniyoshi et al., 2022). Enterolysin A is another class II bacteriocin that is a large and heat-labile protein that inhibits the growth of selected *Enterococci* species (Nilsen et al., 2003). Enterolysin A functions as penocin A by disrupting the cell membranes of the targeted bacteria, leading to their death. This mechanism is pivotal for the competitive advantage of *P. pentosaceus* in various environments (Martino et al., 2013). Both enterolysin A and penocin A highlighted the diverse bacteriocin production potential of ELAB 60WB, which is crucial for its dominance and survival in various environments, including the GIT and fermented foods.

Table 3
Overview of Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology categories of protein-coding genes identified in the *P. pentosaceus* ELAB 60WB genome using BLASTKOALA.

KEGG orthology Number	Functional category	Gene number	Proportion (%)
09101	Carbohydrate metabolism	130	12.43
09102	Energy metabolism	15	1.43
09103	Lipid metabolism	33	3.15
09104	Nucleotide metabolism	57	3.16
09105	Amino acid metabolism	33	3.15
09106	Metabolism of other amino acids	12	1.15
09107	Glycan biosynthesis and metabolism	33	3.15
09108	Metabolism of cofactors and vitamins	34	3.26
09109	Metabolism of terpenoids and polyketides	9	0.85
09110	Biosynthesis of other secondary metabolites	3	0.30
09111	Xenobiotics biodegradation and metabolism	3	0.30
09120	Genetic information processing	155	14.90
09130	Environmental information processing	64	6.12
09140	Cellular processes	11	1.05
09150	Organismal systems	4	0.40
09160	Human diseases	2	0.20
09181	Protein families: metabolism	30	2.86
09182	Protein families: genetic information processing	162	15.40
09183	Protein families: signaling and cellular processes	115	10.99
09191	Unclassified: metabolism	63	6.02
09192	Unclassified: genetic information processing	22	2.10
09193	Unclassified: signaling and cellular processes	14	1.34

3.10. Carbohydrate metabolism

Analysis of carbohydrate-active enzymes (CAZymes) revealed that the ELAB 60WB genome contained 40 genes from the four CAZyme gene families (Fig. 9). These CAZymes are critical for sugar metabolism, including hydrolysis and the formation of glycosidic bonds. ELAB 60WB possessed CAZyme gene families: 1 carbohydrate-binding module (CBMs), 2 carbohydrate esterases, 27 glycoside hydrolase (GH) genes, and 10 glycosyl transferase (GT) genes. GHs are common natural enzymes with the ability to degrade the most abundant biomasses such as starch, cellulose, and hemicellulose (Ameri et al., 2022). A thorough examination of ELAB 60WB differentiation in the presence of GH enzymes focused on GH2, GH13, and GH32, which are recognized as primary oligosaccharide-degrading enzymes (G. G. Gong, Zhou, Luo, Gesang, & Suolang, 2020). Oligosaccharides, which are complex carbohydrates, are the primary sources of prebiotics (Wang et al., 2019; Megur et al., 2022). In addition, dbCAN server analysis showed that ELAB 60WB contained one CBM. CBM enzymes are typically linked to GHs by attaching carbohydrate ligands, which helps improve the catalytic effectiveness of CAZymes (Lu et al., 2024). The two CEs found in ELAB 60WB are involved in the deacetylation of acetylated polysaccharides, which facilitates the subsequent hydrolysis and GHs of carbohydrates (La Rosa et al., 2023). Finally, GT genes in probiotic strains catalyze the transfer of sugar moieties from donor molecules to specific acceptors, making them essential for the biosynthesis of disaccharides, oligosaccharides, and polysaccharides (Al-Emran et al., 2022). The glycoconjugates produced by GTs are important for the formation of bacterial surface structures and facilitate the adhesion of probiotic bacteria to epithelial cells and mucus (Al-Emran et al., 2022). These findings collectively highlight the importance of CAZymes in the metabolic versatility of ELAB 60WB, enabling bacteria to thrive in

Table 4
Genes present in *P. pentosaceus* ELAB 60WB.

Gene	Length (bp)	Function	Response
Heat stress protein			
*hrc*A	1041	Heat-inducible transcription repressor HrcA	Regulates stress response
*hsl*V	555	ATP-dependent protease subunit HslV	Heat-shock response
*hsl*O	915	33 kDa chaperonin	Protects from oxidative stress
*dna*J	1110	Chaperone protein DnaJ	Negatively regulate heat shock gene expression
*dna*K	1860	Chaperone protein DnaK	Maintaining protein homeostasis and ensuring the correct functioning of proteins within the bacterial cell.
*cts*R	465	Transcriptional regulator CtsR	Novel regulator of stress and heat shock response
*grp*E	573	Molecular chaperone GrpE	Heat shock response
*clp*X	1257	ATP-dependent Clp protease ATP-binding subunit ClpX	Mitochondrial unfolded protein response
*clp*E	2220	ATP-dependent Clp protease ATP-binding subunit ClpE	Modulates the gene expression of some virulence factors
Cold stress protein			
*csp*C	210	Cold shock protein CspC	Regulates gene expression and transcript stability of stress proteins and enables cell tolerance to lethal-inducing agents, ultimately enabling adaptation to severe environmental assaults.
*csp*LA	201	Cold shock-like protein CspLA	Regulates gene expression, RNA stability, and membrane fluidity to ensure effective bacterial functioning despite cold environmental conditions.
Acid Stress			
*gad*C	1185	Putative glutamate/gamma-aminobutyrate antiporter	Bacterial survival in acidic environments
*nha*C	1368	Na (+)/H (+) antiporter NhaC	Maintains proper pH and sodium levels within the bacterial cell
*nha*K	1977	Sodium, potassium, lithium, and rubidium/H (+) antiporter	Maintaining the proper balance of sodium and hydrogen ions within the cell
*dna*K	1860	Chaperone protein DnaK	Critical roles in adaptation to environmental stress
desK	1122	Sensor histidine kinase DesK	Membrane fluidity and adaptation to environmental changes in bacteria
Bile tolerance			
*ppa*C	933	Manganese-dependent inorganic pyrophosphatase	Controls pH regulation in bacterial cells
cfa	1176	Cyclopropane-fatty-acyl-phospholipid synthase	Essential for the adaptation and survival of bacteria in diverse environments
Anti-pathogenic effect			
*lux*S	474	S-ribosylhomocysteine lyase	Autoinduction ability
Adhesion			
fbp	1917	Fructose-1,6-bisphosphatase class 3	Adhesion to the mucus layer
*pde*K	681	Putative cyclic di-GMP phosphodiesterase PdeK	Facilitate adhesion to the intestinal epithelium
*Eno*2	1323	Enolase 2	Bacterial adhesion and immunomodulation
Immunomodulation			
*dlt*C	234	D-alanyl carrier protein	Helps maintain cell wall integrity and can influence bacterial resistance to antimicrobial agents and host immune responses
*dlt*D	1287	Protein DltD	Resistance to beta-defensin-2
Antibiotic Resistance			
*tet*A	1176	Tetracycline resistance protein, class B	Resist the effects of tetracycline antibiotics by

(*continued on next page*)

Table 4 (*continued*)

Gene	Length (bp)	Function	Response
			actively pumping the antibiotic out of the cell
*emr*B	1479	Multidrug export protein EmrB	Resistance against multiple antibiotics and other antimicrobial agents
*mep*A	1341	Multidrug export protein MepA	Multidrug resistance
*bmr*3	1467	Multidrug resistance protein 3	Drug resistance
*mdt*D	798	Putative multidrug resistance protein MdtD	Drug resistance
*mdt*L	1176	Multidrug resistance protein MdtL	Multidrug resistance
*lmr*A	1779	Multidrug resistance ABC transporter ATP-binding and permease protein	Multidrug resistance
*pen*A	2091	Penicillin-binding protein 2B	Antibiotic resistance
Amino acid synthesis			
*trp*S2	1029	Tryptophan-tRNA ligase 2	Tryptophan biosynthesis

various environments by efficiently metabolizing a range of carbohydrates.

3.11. Genomic safety assessment

No antimicrobial resistance genes were found in the ResFinder 4.1 database (90% threshold and 60% mini-mum length). No virulence genes were identified in a BLASTn search using VirulenceFinder. A comprehensive tally of 17 virulence genes was predicted using the Virulence Factor of Bacterial Pathogen database, predominantly linked to adherence, stress resilience, iron acquisition, and immune modulation. These genetic elements have been recognized as virulence determinants of pathogens, aiding their persistence within the host milieu, particularly under physiological stress. Interestingly, these attributes may confer advantages to probiotic organisms, enabling their survival within an intricate gut ecosystem (S. Jiang et al., 2021; Zafar & Saier, 2020).

PlasmidFinder identified repUS64 (pR18) in the genome of ELAB 60WB (Supplementary Fig. 2). Antibiotic resistance by this plasmid has been observed in bacteria found in human feces, as it encodes the enzyme linA, which inactivates lincomycin (Jalilsood et al., 2014). This has been previously reported for *P. pentosaceus* DRD-185 and *L. plantarum* DRD-10. This shows how antibiotic resistance traits can spread among different groups of bacteria (Jalilsood et al., 2014). Notably, it has been shown that the repUS64 plasmid has the capacity for interbacterial transfer via conjugation; therefore, this process creates the possibility for new bacteria to acquire antibiotic resistance in the genus of *Lactobacillus* strains (Apostolakos et al., 2023; Jalilsood et al., 2014).

4. Conclusion

This study focused on the isolation, characterization, whole-genome sequencing, and bioinformatic analysis of *P. pentosaceus* ELAB 60WB, a strain derived from fermented cherry tomatoes. The strain exhibited probiotic characteristics and demonstrated antimicrobial activity, resistance to tolerance tests, and safety. Its genome, which consists of 1.9 Mb and comprises 33 contigs, includes genes associated with probiotic survival, with eight identified antibiotic resistance genes indicating intrinsic resistance. Safety assessments revealed the absence of virulence, toxicity, and biogenic amine genes. Plasmid analysis identified repUS64, suggesting its potential for HGT. This strain has considerable potential as a probiotic in the food industry, highlighting the need for further *in vitro* and *in vivo* studies to explore its metabolic and immune modulation applications.

CRediT authorship contribution statement

Ashwinipriyadarshini Megur: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kamilė Ambrutaitytė:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Egidijus Šimoliūnas:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Eglė Lastauskienė:** Writing – review & editing, Visualization, Supervision, Project administration, Data curation. **Aurelijus Burokas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial

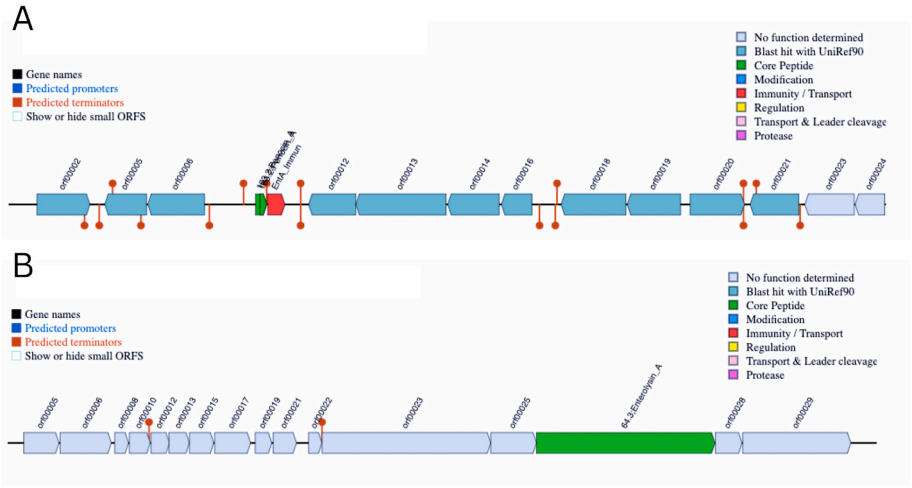

Fig. 8. Organization of bacteriocin gene clusters in the *P. pentosaceus* ELAB 60WB genome was predicted using the BAGEL4 webserver. A) Indication of potential core peptides located on contig 1, B) Indication of potential core peptides located on contig 4.

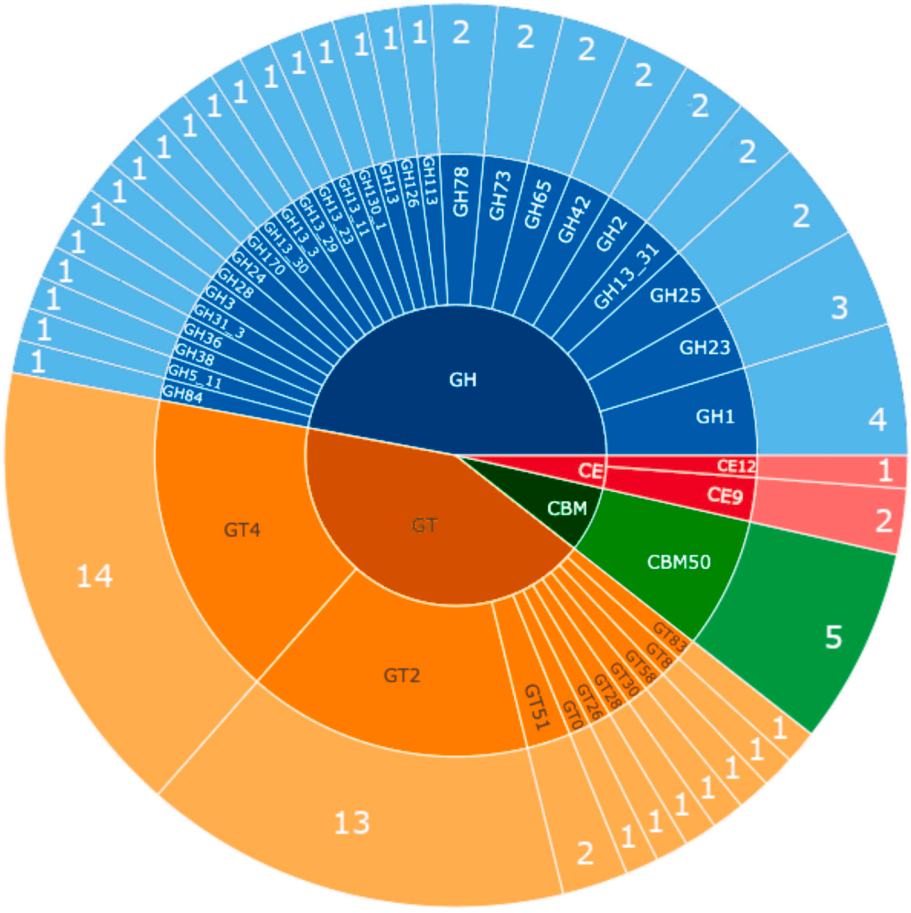

Fig. 9. Distribution of carbohydrate-active enzymes (CAZymes) in *P. pentosaceus* ELAB 60WB. From inner to outer rings, distribution of CAZyme classes, distribution of CAZyme families within each class, and number of ELAB 60WB genes belonging to each family. CBMs – carbohydrate-binding molecule, CE – carbohydrate esterase, GH – glycoside hydrolase, and GT – glycosyl transferase.

interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117547.

Data availability

Data will be made available on request.

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