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IN SILICO ANALYSIS OF THE FUNCTIONAL AND SAFETY ASSESSMENTS OF *LACTIPLANTIBACILLUS PLANTARUM* BBS13 ISOLATED FROM LAO TRADITIONAL FERMENTED BAMBOO SHOOT (*NOR MAI SOM*) BASED ON WHOLE GENOME SEQUENCING

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ABSTRACT

In this study, the genome of strain BBS13 was sequenced using the Illumina HiSeq2500 platform to determine its classification, annotate its main features, and to evaluate its safety characteristics. Genome analysis revealed an average nucleotide identity (ANI) of 99.05% with *Lactiplantibacillus plantarum.* The circular chromosome of BBS13 comprises 3,238,769 bp with a GC content of 44.45%, and a total of 3,054 protein-coding sequences (CDSs) were assigned putative functional roles.

The functional annotation analysis by Rapid Annotation using Subsystems Technology (RAST) integrated with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database revealed that the strain BBS13 possesses genes encoding L-acetate dehydrogenase (*L-LDH*; EC 1.1.1.27) and D-lactate dehydrogenase (*D-LDH*; EC 1.1.1.28), which are responsible for lactic acid production, as well as linamarase or β-glucosidases (EC 3.2.1.21). Interestingly, through the safety assessments via WGS, no virulence factors, biogenic amines, or antimicrobial resistance genes was found in this strain. Our previous studies showed probiotic properties of this strain such as tolerance to the simulated artificial gastrointestinal tract, bacterial adhesion, antibacterial activity, and antioxidant function. The findings presented herein greatly boosted the level of information available on BBS13 in support of its potential application in food products for health promotion.

Keywords: *Lactiplantibacillus plantarum* BBS13, Safety assessment, Whole-genome sequencing (WGS) analysis

INTRODUCTION

Lactiplantibacillus plantarum belongs to the family *Lactobacillaceae* in the order *Lactobacillales* **(Lv *et al*., 2014 and Zheng *et al*., 2020).** Due to their ability to produce antimicrobial compounds such as acidophilucin A, lactacins A, pediocin, plantaricin, nisin Z, and reuterin, together with their documented probiotic attributes, including resistance to gastric acid and bile salts, adhesion capacity, antibacterial activity, antibiotic susceptibility, absence of biogenic amine production and virulence factors, and non-hemolytic behavior. These microorganisms are considered non-pathogenic and have a well-established history of safe use as starter cultures in the food and beverage industry **(Lv *et al*., 2014).** The development of food products that provide health advantages beyond just basic nutrition is an outcome of a greater awareness of probiotic microorganisms. Several disparate and usually strain-specific studies have been made on the beneficial effects of probiotic bacteria **(Anjana and Tiwari, 2022).**

Numerous studies have demonstrated that LAB exhibit a wide range of health-promoting bioactivities, including enhanced lactose metabolism, anti-inflammatory effects, immune system modulation, prevention of gastrointestinal infections, as well as anticancer, antioxidant, antibacterial, cholesterol-lowering, and antihypertensive properties. These functional attributes highlight the potential application of probiotic bacteria in biomedical and pharmaceutical fields **(Gezginç *et al*., 2022).** *L. plantarum* has been widely employed in food fermentation due to its safe characteristics and enhancement of the properties of fermented products. Some strains have been demonstrated to produce diverse antimicrobial substances such as bacteriocins, organic acids, hydrogen peroxide, and diacetyl. The application of *L. plantarum* in the food industry as a prospective biocontrol strategy against pathogenic microorganisms demands emphasis. Moreover, *L. plantarum* represents a more sustainable alternative to artificial antibacterial agents and exhibits significant potential in the advancement of functional foods **(Yilmaz *et al*., 2022). Shi *et al*. (2024a)** stated that *L. plantarum* has emerged as a promising alternative for food bio-preservation due to its ability to function as a natural antibacterial and anti-virulence agent. This capability is attributed to the production of diverse antimicrobial metabolites and competitive exclusion mechanisms. Consequently, there is growing interest from both producers and consumers in developing innovative "clean-label" preservation strategies for a wide range of food products. Another investigation by **Shi *et al*. (2024b)** addressed the new trends of LAB in fermented herb-derived food products (FHFP), a new type of fermented food in which LAB plays a role in the production of new active constituents, as well as the improvement of functionality, bioavailability, quality, and added value of FHFP. Probiotic foods are a significant class of functional foods that are available on the global market and have successfully achieved a market value of US $46.55 billion in 2020 **(Singh *et al*., 2018; Tarrah *et al*., 2020).** Since many LAB are generally recognized as safe (GRAS), they can thus be recommended for use in food products on the market without the need for deep investigation to demonstrate their safety. The probiotic potential of several LAB, including those from the genera *Bifidobacterium*, *Pediococcus*, *Lactococcus*, and *Enterococcus*, have been investigated recently. The majority of probiotics currently available on the market are from the genus *Lactobacillus*, a taxonomically heterogeneous group that contains more than 170 species **(Tarrah *et al*., 2020).**

In recent years, the accessibility of next-generation sequencing (NGS) such as whole-genome sequencing (WGS), has led to a significant increase in the number of sequencing-based data to characterize the systematics and molecular taxonomy in detail, enzyme system, and bioactive compounds of LAB species **(Kwong *et al*., 2015; Arjun *et al*., 2024).** The complete genome sequences of various *L. plantarum* have been analyzed by numerous studies **(Midha *et al*., 2012; Lv *et al*., 2014; Chokesajjawatee *et al*., 2020; Qureshi *et al*., 2020; Kwon *et al*., 2021; Carpi *et al*., 2022),** allowing new approaches to be introduced to deduce the evolutionary and divergent relationships among the strains.

WGS is a reliable technique for precisely characterizing strains and interpreting LAB functions at the genomic level **(Sharma *et al*., 2020).** Moreover, the starter cultures utilized for food fermentation have rarely been as extensively evaluated for safety. The need for assessing the safety of microorganisms used in food has long been recognized **(Chokesajjawatee *et al*., 2020).** The European Food Safety

Authority **(EFSA, 2012)** published guidance for evaluating the safety of probiotics, stating that living probiotic bacteria need to be characterized for safety before being applied to food products. These bacteria isolated from various sources should not carry any toxic substances and factors (biogenic amines, virulence factors, resistance genes), and should be both non-pathogenic and unaffected by diseases (infective endocarditis or digestive tract disorders) **(Gao *et al*., 2014; Dlamini *et al*., 2019). Pariza *et al*., (2015)** recommended whole-genome analysis as part of strain identification and safety evaluation, which is gaining more interest. With WGS analysis, safety assessments of bacterial strains can be conducted at a much greater level of detail. Therefore, this study was conducted to: (1) sequence *L. plantarum* BBS13 strain, isolated from *Nor Mai Som*, a traditional Lao fermented bamboo shoots product; (2) ascertain its classification and perform the annotation of the genome' s main features; and (3) evaluate the safety characteristics (virulence factors, synthesis of biogenic amines, presence of antimicrobial resistance (AMR) genes, and bacterial mobile genetic elements) of the strain via WGS.

MATERIALS AND METHODS

Bacterial Strains and Growth Media

Lactiplantibacillus plantarum BBS13 (PRJNA937409) was isolated from Lao traditional fermented bamboo shoots. The strain used in this study was maintained as stock cultures at- 80ºC in 30% glycerol until further use.

Whole Genome Sequencing Analysis

After ensuring the results from the previous study that strain BBS13 produced antibacterial compounds, the desired enzymatic action, and probiotic features, as well as phenotypic functional and safety assessments **(Botthoulath *et al*., 2024).** The strain BBS13 was selected for whole genome sequencing analysis. The strain was grown in MRS medium (HiMedia, India) for 18-24 h at 37ºC and stored as stock culture at -20ºC in the presence of 30% (v/v) glycerol. Prior to use in the different experiments, the isolate was sub-cultured at least twice in MRS broth for 18-24 h at 37ºC.

Genomic DNA Extraction

Genomic DNA extraction was performed following the protocol described by **Botthoulath *et al.*** (2018) with slight modifications. Bacterial cultures were harvested during the early exponential growth stage to ensure optimal DNA yield. DNA extraction was carried out using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research Corp., USA) in accordance with the manufacturer's guildelines. The yield and quality of the extracted DNA were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). DNA purity was evaluated based on the absorbance ratio at 260 and 280 nm, with acceptable values ranging from 1.8 to 2.0 (**Chokesajjawatee *et al*., 2020**).
The quantity and integrity of the extracted genomic DNA were evaluated by agarose gel electrophoresis to assess the yield of genomic DNA. DNA samples were mixed with 4X loading dye at a ratio of 3:1 and loaded onto a 1% (w/v) agarose gel supplemented with GelRed™ nucleic acid stain (Zymo Research Corp., USA). Following electrophoresis, DNA bands were visualized under ultraviolet illumination to confirm successful extraction. Samples exhibiting clear and intact bands were subsequently selected for whole-genome sequencing.

Genome Sequencing and Genome Assembly

The genomic DNA of strain BBS13 was submitted for whole genome sequencing using Illumina technology (Illumina Inc., Macrogen, Korea). The sequencing library was prepared using TruSeq kits and the library QC with the standard PacBio library protocol. Paired-end reads with 100 bp setting was produced using a HiSeq2500 platform sequencing instrument. Raw sequencing data in BAM, SAM, or FASTQ formats were assessed for quality using FastQC v0.11.7 both prior to and following read trimming to ensure data reliability **(Andrews, 2010).** Trimmomatic v0.3833 was used to trim reads (including adapter removal) and reject sequences having a pair base sequence quality score <30 **(Bolger *et al*., 2014).** High-quality reads were subsequently assembled de novo using Unicycler v 0.4.7 (**Wick *et al*., 2017**). In parallel, genome assembly was also conducted through the PATRIC platform (v3.6.9) using default settings (**Davis *et al*., 2020**), available at the PATRIC Bioinformatics Resource Center, https://www.patricbrc.org/

Gene Prediction and Functional Annotation

Prediction of protein-coding genes and functional annotation were conducted to characterize the genomic features of strain BBS13. This analysis was carried out using the Rapid Annotation using Subsystems Technology (RAST) server (**Aziz *et al*., 2008),** accessible at https://rast.nmpdr.org/ with default settings. To visualize the genome organization, a circular genome map of strain BBS13 was generated using the Bacterial and Viral Bioinformatics Resource Center (BV-BRC, v3.29.20) through the Pathosystems Resource Integration Center (PATRIC) tools (**Brettin *et al*., 2015**), accessible at https://www.bv-brc.org/

Identification of Species

The taxonomic identification of strain BBS13 was determined using a combination of 16S *rRNA* gene sequence analysis and average nucleotide identity (ANI), following the general approach described by **Chokesajjawatee *et al*. (2020).** The 16S *rRNA* gene sequence was retrieved from the assembled genome and screened for potential contamination using the web-based ContEst16S tool (**Lee *et al*., 2017**). Species-level identification was further validated by ANI analysis conducted with the Orthologous Average Nucleotide Identity Tool (OAT) (**Lee *et al*., 2016).** An ANI threshold of 95–96% was applied as the cutoff for confirming species delineation, in accordance with established criteria (**Richter and Rosselló-Móra, 2009**).

Bioinformatic Analysis of Safety Assessments of *Lactiplantibacillus plantarum* BBS13

Detection of virulence factors, biogenic amine, and antimicrobial resistance genes via whole genome analysis

Potential virulence factors genes were screened using a stringent search strategy with threshold criteria set at >80% sequence identity and >60% query coverage. The genome of strain BBS13 was examined against the Virulence Factor Database (VFDB; last updated June 17, 2019) to identify putative virulence factors and toxin-related genes (**Liu *et al*., 2019**), http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi. In addition, OriTfinder (v1.1; **Li *et al*., 2018**), available at https://bioinfo-mml.sjtu.edu.cn/oriTfinder/ (database v1.1, May 2017) and the Rapid Annotation using Subsystems Technology (RAST) integrated with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (**Overbeek et al., 2014**), available at https://rast.nmpdr.org/rast.cgi, were employed as complementary tools to detect virulence factors and other undesirable genes, in accordance with the recommendations of the European Food Safety Authority (**EFSA, 2011**).
Biogenic amine–related genes, including arginine, cadaverine, histamine, ornithine, putrescine, spermidine, tyramine, and tryptamine, were investigated within the genome of strain BBS13. This analysis was performed using the RAST platform in conjunction with KEGG pathway mapping, available at https://rast.nmpdr.org/rast.cgi.
The presence of antimicrobial resistance (AMR)–associated genes in the genome of strain BBS13 was assessed using multiple publicly accessible databases. Genome sequences were screened against ResFinder (v4.1; database updated July 19, 2019) (**Zankari *et al*., 2010**), available at https://cge.cbs.dtu.dk/services/ResFinder/, and the Comprehensive Antibiotic Resistance Database (CARD) using the Resistance Gene Identifier (RGI version 5.0.0; CARD v3.0.3) (**Alcock *et al*., 2023**), available at https://card.mcmaster.ca. In addition, AMR genes were further examined through the RAST platform linked to KEGG) pathways, accessible at https://rast.nmpdr.org/rast.cgi.
Antimicrobial resistance genes associated with mobile genetic elements, including conjugative plasmids, plasmids, and intact prophages, were further examined to evaluate their potential for horizontal transfer. The identification of origin-of-transfer (oriT) sites, which indicate the capacity for self-mediated conjugative transfer, was carried out using the web-based OriTfinder tool (v1.1; **Li *et al*., 2018**), accessible at https://bioinfo-mml.sjtu.edu.cn/oriTfinder/

RESULTS AND DISCUSSION

Lactiplantibacillus plantarum BBS13 Whole Genome Sequencing and its main Genomic Features

Strain BBS13's whole genome was likewise sequenced on Illumina HiSeq2500 platform. **Table 1** describes the main features of the *L. plantarum* BBS13 genome consisting of a circular chromosome with 3,238,769 bp and a GC content of 44.45%. The RAST server's gene prediction and annotation revealed that a total of 3,054 protein coding sequences (CDSs) were allocated to putative functions, which were distributed all over 332 subsystems. A total of 102 contigs, 71 *tRNA* genes, and 7 *rRNA* genes were predicted. **Figure 1a** displays attributes such as CDS on the forward and reverse strands, GC content, and GC skew on a circular graphical map, which was conducted using Patric v3.6.9. **Figure 1b** demonstrates the number of subsystem components typically responsible for a particular biological process or structural complex. The majority of this subsystem's feature counts were attributable to carbohydrate metabolism (423) and followed by protein metabolism (186). This suggests that this strain possesses a highly remarkable carbohydrate metabolism capacity. **Yang *et al*. (2022)** determined the complete genome sequence of *L. plantarum* ST isolated from De'ang pickled tea. The results indicated that the circular chromosome had 3,058,984 bp and a GC content of 44.76%. It contained 2,945 CDS, the majority of which were involved in carbohydrate transport and metabolism, which was consistent with the subsystem annotation results in this study. Similar investigation by **Mao *et al*. (2021)** revealed

that the number of coding genes associated with carbohydrate metabolism was the greatest followed by protein metabolism, indicating that this strain has a remarkable potential for carbohydrate metabolism as well as providing molecular support for the metabolism of various amino acids. Microorganisms grow and develop using carbohydrates and proteins. For example,

LAB can adapt by acquiring carbohydrates from the environment or eliminating carbohydrate genes **(Yang *et al*., 2022).** Also, *L. plantarum* BBS13 found subsystem feature annotations on the cell wall and capsule (126), which shows that this strain is able to form biofilms, making it more resistant to external harmful substances or other damaging factors. Strain BBS13 genome, therefore, provides a scientific rationale for its potential use in fermented foods **(Yang *et al*., 2022).**

Table 1 Genome features of *Lactiplantibacillus plantarum* BBS13 analyzed by Rapid Annotation using Subsystems Technology (RAST)

	Values
Genome size (bp)	3,238,769
GC content (%)	44.45
Contig N50	118,864
Contig L50	9
Number of contig	102
Number of protein coding sequences (CDSs)	3,054
Number of subsystems	332
tRNA	71
rRNA	7

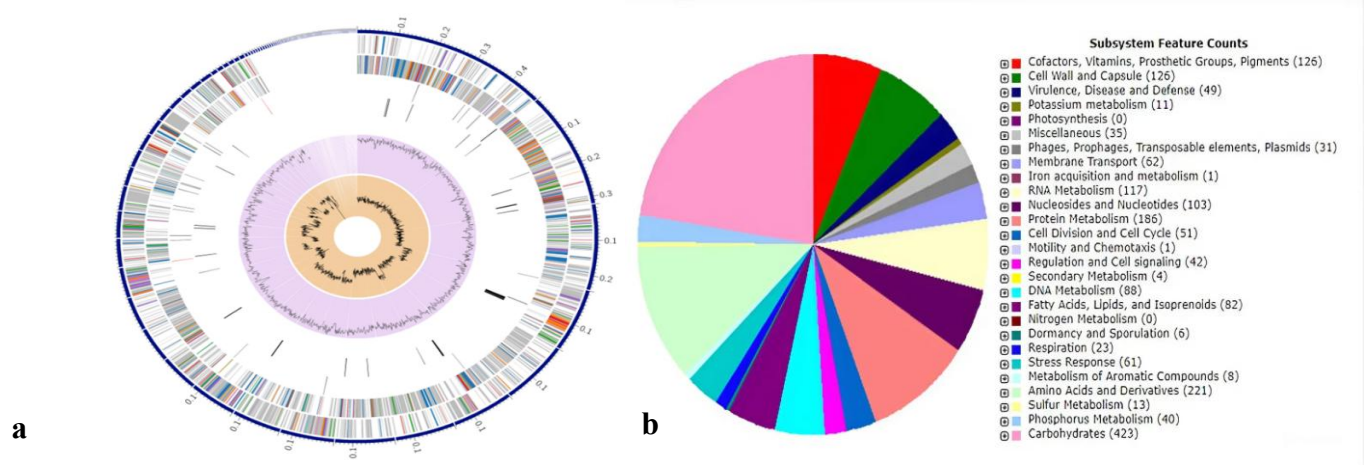

Figure 1 A circular graphical genome map of *Lactiplantibacillus plantarum* BBS13 analyzed by the Pathosystems Resource Integration Center (PATRIC) tool-Bacterial and Viral Bioinformatics Resource Center (BV-BRC 3.29.20). From outer to inner rings, the contigs, protein coding sequences (CDSs) on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to known virulence factors, GC content, and GC skew (a). A description of the Rapid Annotation using Subsystems Technology (RAST) linked annotation and related subsystems for this gene. The colors of the CDS on the forward and reverse strands show the subsystem that these genes belong to (b).

Identification of Species by Whole genome Sequencing (WGS)
***Lactiplantibacillus plantarum* BBS13**

Strain BBS13 was previously identified as *L. pentosus or L. plantarum* based on its 16S *rRNA* gene sequence. Nonetheless, the analysis solely based on the 16S *rRNA* gene was insufficient to differentiate between *L. plantarum* and *L. pentosus,* as these species are genotypically closely related and exhibit shared highly homologous phenotypes **(Torriani *et al*., 2001).** The WGS analysis was then utilized to guarantee that these species' distinctions were effectively resolved (**Figure 2**). According to the ANI analysis, strain BBS13 was 99.05% highly conserved in comparison to those of *L. plantarum* WCFS1 (PRJNA858558), *L. plantarum* strain SRCM100442 (NZ_CP028221.1), and *L. plantarum* BCC9546 (EU391630.1). *L. pentosus* strain DSM 20314 (NZ_CP032757.1) was established as an outgroup (<95-96% cut-off threshold). Strain BBS13 demonstrated values of 0.01 against all *L. plantarum* strains in a Genome-to-Genome Distance Calculator (GGDC) analysis. The present study is in agreement with the study by **Chokesajjawatee *et al*. (2020),** which reported that *L. plantarum* and *L. pentosus* cannot be distinguished based simply on the 16S *rRNA* gene. The result from WGS identification showed the accuracy of differentiating these species, with *L. plantarum* BCC9546 sharing the highest ANI value of 98.74% to *L. plantarum* ATCC 1491^{T}. Similarly, **Carpi *et al*. (2022)** and **Yang *et al*. (2022)** identified *L. plantarum* strains based on the genome sequence derived from ANI analyses. Thus, the whole-genome data proved that strain BBS13 belonged to the species *L. plantarum* (BioProject accession number of strain BBS13 is PRJNA937409).

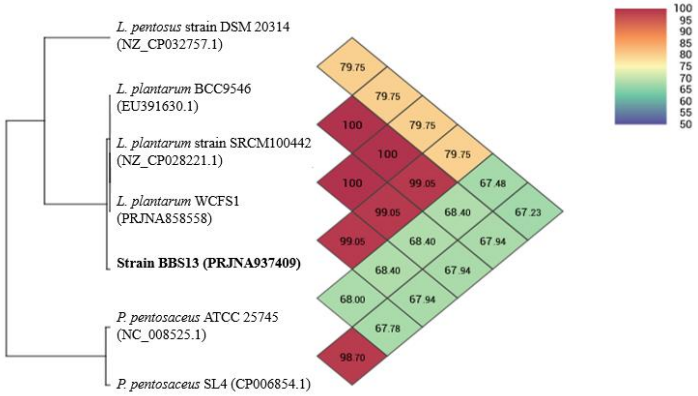

Figure 2 Using the Orthologous Average Nucleotide Identity Tool (OAT), the whole genome sequence of the strain *Lactiplantibacillus plantarum* BBS13 was compared with other reference strains in GenBank (access numbers in parenthesis). The OrthoANI calculation of strain BBS13 had the highest ANI value of 99.05% to *Lactiplantibacillus plantarum* WCFS1 (PRJNA858558), and *Lactiplantibacillus plantarum* strain SRCM100442 (NZ_CP028221.1).

Lactic Acid Production

Microbial fermentation has traditionally produced lactic acid, which is employed in the food, pharmaceutical, cosmetic, and chemical industries such as bioplastics from the green polymer poly-lactic acid (PLA) **(Li *et al*., 2013).** Enzyme-catalyzed microbial lactic acid production is more pure than chemical synthesis. Microbial fermentation is preferred for lactate synthesis because only optically pure L- and D-lactic acid monomers can be utilized as PLA precursors **(Zheng *et al*., 2012; Ma *et al*., 2014).** Analysis using the RAST tool linked to the KEGG pathway revealed L-lactate dehydrogenase (*L-LDH*; EC 1.1.1.27) and D-lactate dehydrogenase (*D-LDH*; EC 1.1.1.28), the genes responsible for lactic acid production in *L. plantarum* BBS13 genomes (**Table 2**). It is evident that the ldhA, D-lactate dehydrogenase (EC 1.1.1.28) gene sequence was present in three positions with varying sizes were observed in BBS13 genome (993 bp, 1191 bp, and 999 bp). For the *LDH, ldh*; L-lactate dehydrogenase (EC 1.1.1.27) in BBS13 was contained six positions with lengths of 963 bp, 927 bp, 960 bp, 930 bp, 933

bp, and 918 bp. Similar findings had been reported earlier by **Chokesajjawatee *et al*. (2020),** who discovered that the two genes, lactate racemase (chr 00083) and D-lactate dehydrogenase (chr_00684 and chr _1677), were determined to be responsible for the formation of D-lactic acid after searching through the KEGG database. Zheng *et al*. (2012) showed that the optical purity of the lactic acid produced by *Lactobacillus* strains depend on the respective catalytic efficacy of *ldhL*- and *ldhD*-encoded products. In LAB, *L-LDH* and *D-LDH* exhibit a wide range of catalytic characteristics and play essential roles in the fermentation of lactic acid, participating in the final stage of the anaerobic glycolysis pathway by converting pyruvate and NADH to L- and D-lactic acid, respectively **(Arai *et al*., 2001; Sun *et al*., 2016).** This current research has shown that the genes capable of producing lactic acid are present in BBS13. This was corresponding with our previous studies on phenotypic analysis of lactic acid production; the determination of homofermentative LAB producing organic acids and high-performance liquid chromatography (HPLC) analysis were examined in parallel to ensure lactic acid production of BBS13 during the *Nor Mai Som* fermentation process **(Botthoulath *et al.*, 2024),** thereby providing an advantage when applied to food and other related products. Also, the presence of these genes may be used as a guide for enhancing the fermentation efficiency of lactic acid production through genetic manipulation with plasmid transformation.

Table 2 List of the D-lactate and L-lactate formation of *Lactiplantibacillus plantarum* BBS13 analyzed by using the Rapid Annotation using Subsystems Technology (RAST) linked to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway

Feature ID	Gene ID	Type	Coordinates	Strand	Length (bp)	Function
D-lactate formation						
fig|1590.3478.peg.2147	K03778	CDS	Start: 35270 Stop: 34278	-	993	ldhA; D-lactate dehydrogenase (EC 1.1.1.28)
fig|1590.3478.peg.2148	K03778	CDS	Start:36463 Stop: 35273	-	1191	ldhA; D-lactate dehydrogenase (EC 1.1.1.28)
fig|1590.3478.peg.2467	K03778	CDS	Start: 162988 Stop: 161990	-	999	ldhA; D-lactate dehydrogenase (EC 1.1.1.28)
L-lactate formation						
fig|1590.3478.peg.130	K00016	CDS	Start: 25677 Stop: 24715	-	963	LDH, ldh; L-lactate dehydrogenase (EC 1.1.1.27)
fig|1590.3478.peg.1394	K00016	CDS	Start: 90322 Stop: 89396	-	927	LDH, ldh; L-lactate dehydrogenase (EC 1.1.1.27)
fig|1590.3478.peg.1469	K00016	CDS	Start: 17519 Stop: 18478	+	960	LDH, ldh; L-lactate dehydrogenase (EC 1.1.1.27)
fig|1590.3478.peg.1756	K00016	CDS	Start: 158786 Stop: 159715	+	930	LDH, ldh; L-lactate dehydrogenase (EC 1.1.1.27)
fig|1590.3478.peg.2547	K00016	CDS	Start: 247094 Stop: 246162	-	933	LDH, ldh; L-lactate dehydrogenase (EC 1.1.1.27)
fig|1590.3478.peg.2958	K00016	CDS	Start: 45415 Stop: 46332	+	918	LDH, ldh; L-lactate dehydrogenase (EC 1.1.1.27)

Linamarase

Linamarase or β-glucosidase enzyme (EC 3.2.1.21) commonly exists in microorganisms, especially in LAB **(Xie *et al*., 2022).** Therefore, the presence of linamarase genes in the genomes of BBS13 was analysed using the RAST tool through the KEGG pathway. **Figure 3** indicates the presence of β-glucosidase in the BBS13 genome, with a DNA sequence of 1386 bp and 462 amino acids. It exhibited a 100% similarity in β-glucosidase amino acid sequences to those associated with the *L. plantarum* WCFS1 genome (reference strain) (**Figure 4a**), as aligned by UniProt, accessible at https://www.uniprot.org/. The comparison of β-glucosidase amino acid sequences of these strains was precisely illustrated in the amino acid sequence alignment using the CLC Sequence Viewer 8.0 tool (**Figure 4b**). In relation to the earlier studies, **Michlmayr and Kneifel, (2014)** reviewed the putative β-glucosidase and phospho-β-glucosidase genes of *L. plantarum* WCFS1 and their predicted organization in operons were analyzed. This information was obtained from the prokaryotic operon database **(Taboada *et al*., 2012). Xie *et al*. (2022)** reported that the β-glucosidase was analyzed by complete genome sequencing in the *L. paracasei* TK1501 isolated from naturally fermented congee, and the β-glucosidase amino acid sequence from *L. paracasei* TK1501 had a 99.8% similarity to that of *L. casei* ATCC334 (reference strain). Theoretically, β-glucosidases (EC 3.2.1.21) hydrolyze glycosidic linkages and remove glucopyranosyl residues from the nonreducing end of arylglucosides, alkylglucosides, cellobiose, and cellooligosaccharides. This enzyme's substrate specificity, inducers, and cellular location differ widely among various microbial enzymes **(Lei *et al*., 1999; Fadahunsi *et al*., 2020; Michlmayr and Kneifel, 2014).** β-glucosidases are utilized in the food industry to liberate aromatic compounds from glucoside precursors and provide preferable flavor and aroma in fruits and fermented products. The β-glucosidase activities of several LAB, including *L. plantarum*, *L. pentosus*, *L. brevis*, *P. pentosaceus*, *Leuconostoc*, and *Weissella* are utilized to remove the bitterness of linamarin, a toxic cyanogenic glucoside **(Nout and Sarkar, 1999; García-cano *et al*., 2020).** Therefore, based on genomic information, the existence of β-glucosidase genes in BBS13 was revealed function of the enzyme features. This is consistent with previous investigations on cyanide resistance and linamarase activity, which found that strain BBS 13 was able to grow in potassium cyanide (800 mg/L, KCN) solution and had linamarase activity, an enzyme that is capable of breaking down cyanide into basic substances that bacteria can use for metabolism and growth **(Botthoulath *et al*., 2024).** Thus, strain BBS13 provided a much-needed benefit to the food industry, particularly their use as starter cultures with the purpose of reducing cyanide content. They may also pave the way for genetic engineering techniques that use plasmid transformation to boost linamarase production in food fermentation.

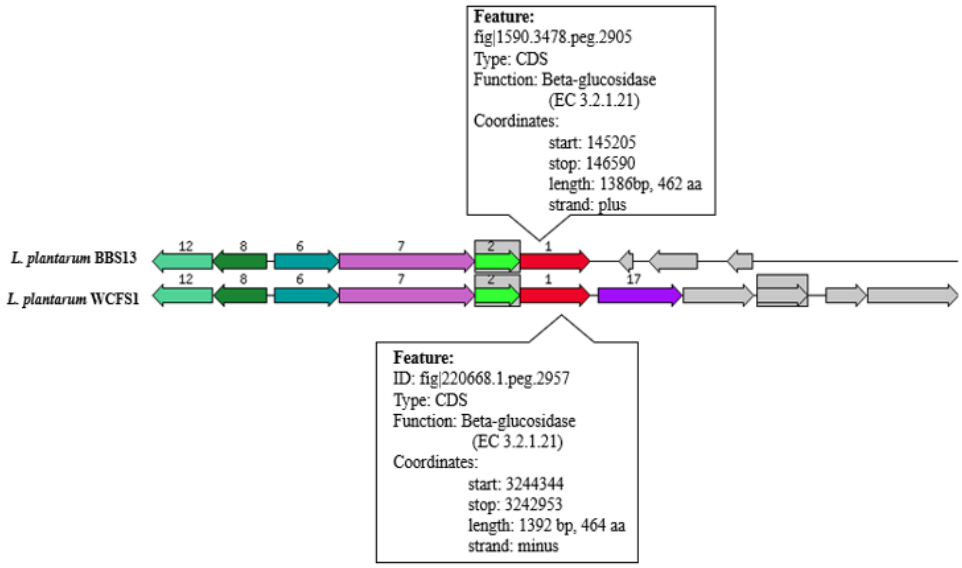

Figure 3 Comparison of the chromosomal gene regions for beta-glucosidase (EC 3.2.1.21) of *Lactiplantibacillus plantarum* BBS13 with other reference strains, analyzed by Rapid Annotation using Subsystems Technology (RAST) linked to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. The graphic is centered on the focus gene, which is red and numbered 1 (included in gray background boxes), genes that probably share other functional features.

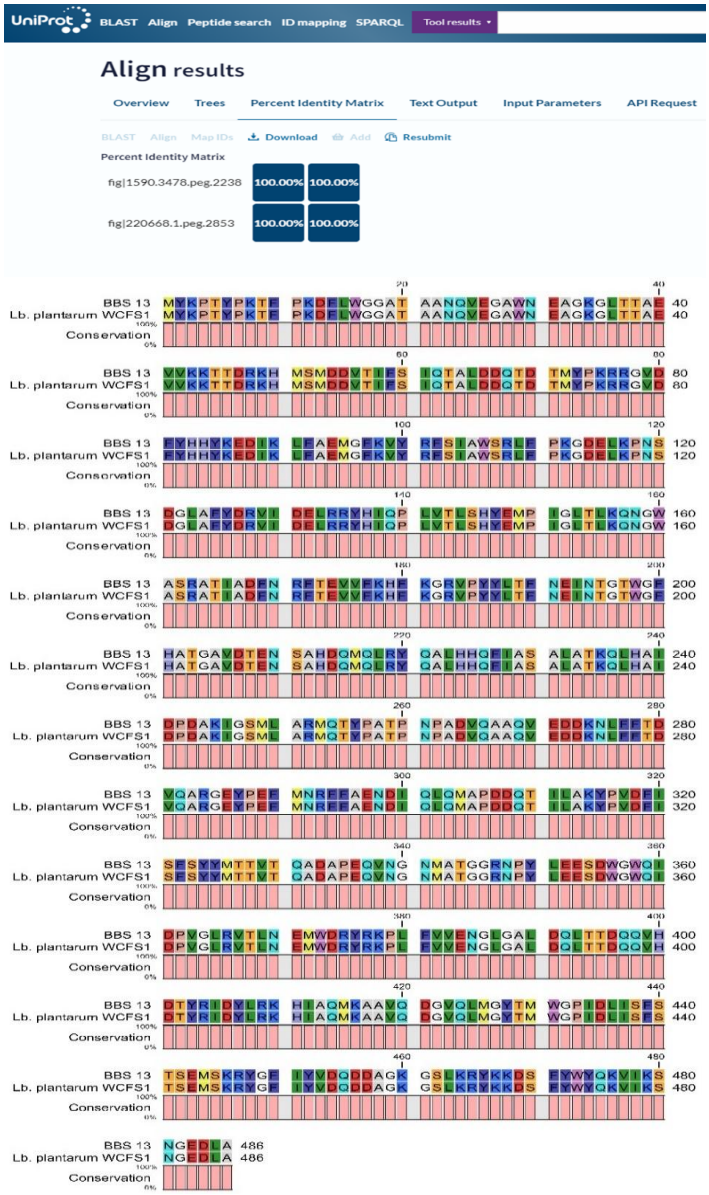

Figure 4 *Lactiplantibacillus plantarum* BBS13 showed 100% similarity of β-glucosidase amino acid sequences to the *Lactiplantibacillus plantarum* WCFS1 genome by using the UniProt tool (a). Comparison of β-glucosidase amino acid sequences of *L. plantarum* BBS13 against the reference strain *L. plantarum* WCFS1 by using the CLC Sequence Viewer 8.0 tool (b).

Whole Genome Analysis on the Safety Concerns in *Lactiplantibacillus plantarum* BBS13

Detection of Virulence Factor Genes

As a foundation for probiotic safety research, a framework for evaluating candidates for safe probiotic LAB should be derived. Because safety is an utmost priority, characterization of strain BBS13 should include the identification of potentially unwanted properties. The evaluation of virulence genes and antimicrobial resistance genes from reputable databases, such as the Virulence Factors Database (VFDB) and the Antibiotic Resistance Genes Database (ARDB), for individual probiotic candidates, is essential for in silico risk assessments of strain BBS13, which intends to be used as a probiotic source in food sections in the future **(Salvetti *et al*. 2016).** It is necessary to address the possibility of genetic factors encoding these genes being transferred to other organisms. The WGS is one strategy for addressing these concerns for every strain that is incorporated in the product **(Peng *et al*., 2023).**

Virulence is one of the criteria considered when selecting probiotic strain BBS13 for use as a starter culture and as a probiotic in food products. Due to the presence of encoding genes in specific regions of the genome that are capable of causing diseases (nosocomial illnesses, severity of endocarditis, intestinal and extraintestinal, inflammatory or enterococcal), this trait has gained considerable attention in recent years **(Vesterlund *et al*., 2007; Pakbin *et al*., 2021).** Screening of BBS13 genome using VFDB revealed no detectable virulence-associated genes when applying stringent thresholds cut-off of >80% sequence identity and >60% query coverage (**Table 3**). Additional analysis using OriTfinder identified the presence of *clpP, hasA, hasB, hasC*, and *bsh* genes. These genes are associated with stress tolerance, immune interaction, and bile salt hydrolysis, respectively, and are not indicative of pathogenic potential. Similar patterns were detected under RAST linked to KEGG via "Brite" genes and proteins for the *bsh* gene, whereas *YqfA* was absent (encoding for Hemolysin III family). Recent studies on the safety of probiotic LAB based on WGS have been performed by numerous authors. Related to the study by **Zhang *et al*. (2012),** which used the VFDB database and various stringency criteria to identify virulence factors in *L. plantarum* JDM1, the results showed the presence of 126 virulence-associated genes, while the hemolysin III gene was absent. Compared to **Chokesajjawatee *et al*. (2020),** a safety assessment based on WGS analysis in *L. plantarum* BCC9546 used as a starter culture in *Nham* product indicated no virulence factors under the cut-off standard values. Hemolysin III (chr 02698) was the only toxin gene found in 51 hits using less stringent criteria. Another study by **Syrokou *et al*., (2022),** which employed WGS to assess the safety of six strains of *L. plantarum* isolated from spontaneously fermented Greek wheat sourdoughs, revealed the absence of pathogenic factors through VFDB database. However, the majority of genes were identified as defensive or non-classical virulence factors, while none were offensive virulence factors. The results of numerous studies raise awareness of the need for consensus criteria or a standardized protocol for the optimal process of safety assessment **(Zhang *et al*. 2012).** The none-offensive virulence factors found in this current study using other tools (OriTfinder and RAST linked to KEGG database), were engaged in the adaptation, survival, or attachment of pathogenic

bacteria to their hostile/host environment. Moreover, with the lack of other pathogenic factors, these genes can be considered advantageous to the bacterium because they promote bacterial fitness and may be beneficial in conditions where viable cells are required, particularly if used as starter cultures and also serve as probiotics. Consequently, based on the findings of this present research, it was resolved that the search using multiple tools was accurate and effective for its intended purpose, and that *L. plantarum* BBS13 do not present any safety issues related to their virulence factor.

Table 3 Detection of virulence factor genes analyzed by virulence factor database (VFDB) and by Rapid Annotation using Subsystems Technology (RAST) linked to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway in *Lactiplantibacillus plantarum* BBS13

***L. plantarum* BBS13**				
Virulence factor category	**Gene ID/Accession number**	**Related gene**	**Product/function**	**Result**
Under virulence factor database (VFDB)			Not found	Not found
Under OriTfinder				
Stress survival	lmo2468 NP_465991	*ClpP*	- ATP-dependent *Clp* protease proteolytic subunit. - Serine protease involves proteolytic enzyme essential for bacterial growth and adaptation during stressful environmental conditions **(Gaillot *et al*., 2001**).	Found
Immune modulation; Antiphagocytosis	SPY_RS09095 WP_010922799	*hasA; hasB; hasC*	- UTP-glucose-1-phosphate uridylyltransferase *hasC.* - GAS capsular hyaluronate chemically resembles human connective tissue. Consequently, this capsule inhibits C3b deposition, allowing the bacteria to evade immune recognition by mimicking host structures and thereby resisting phagocytosis **(Ashbaugh *et al*., 1998**).	Found
Bile salt hydrolysis/ Stress survival	lmo2067 NP_465591	*bsh*	- Bile salt hydrolase. - This factor is essential for the intestinal persistence of *L. monocytogenes* and contributes to resistance against the acute toxic effects of bile and bile salts **(Begley *et al*., 2005).**	Found
Under RAST linked to KEGG via "Brite" Genes and Proteins				
Bile hydrolysis	K01442	*bsh*	- Choloylglycine hydrolase (EC 3.5.1.24). - Bile salt hydrolysis is a crucial step in fat metabolism **(Ren *et al*., 2011).**	Found
Hemolysin III family	K11068	*YqfA*	- Predicted membrane channel-forming protein *YqfA.*	Not found

Detection of Biogenic Amine Genes (BAs)

Biogenic amines (BAs) are of issue in fermented food because of their toxic effects on the nervous system, hypertension, and digestive system **(Swetwiwathana et al., 2015).** Many BAs have been detected in *Enterococcus, Lactobacillus, and Pediococcus spp*., which are important BA producers in fermented foods. Strain BBS13, which may be intended for use as a probiotic source in the future, particularly *Nor Mai Som* or related fermented products. As a result, this strain should be examined for potential BA safety concerns.

Strain BBS13 genome did not contain any biogenic amine genes, including those for arginine, cadaverine, histamine, ornithine, putrescine, spermidine, spermine, tyramine, and tryptamine, as determined by the RAST analysis via KEGG pathways **(Table 4).** This is consistent with the study by **Chokesajjawatee *et al*. (2020)** which reported that no genes related to the production of BA were found in *L. plantarum* BCC9546. **Oliveira *et al*. (2022),** reported that genes related to biogenic amines were not detected. In general, the amounts of BA found in food products depend on several factors, including microbiota quality and quantity, chemico-physical variables, fermentation hygiene, precursor amino acids, pH, temperature, and son on **(Swetwiwathana *et al*., 2015).** As recommended by EFSA, a bacterial strain that lacks the genes necessary for BA formation is known as a BA-nonproducing strain and is considered non-harmful. If the genes were discovered, the actual production and accumulated BA levels at the intended usage conditions should be established to assess the risk **(Chokesajjawatee *et al*., 2020).** In accordance with the findings of the bioinformatics investigation made in the present study, *L. plantarum* BBS13 can be described as non-producers of BA and pose no safety risk.

Table 4 Detection of biogenic amine genes analyzed by the Rapid Annotation using Subsystems Technology (RAST) linked to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway in *Lactiplantibacillus plantarum* BBS13

BBS13				
Name	**Gene ID**	**Enzyme name**	**product**	**Result**
Biogenic amine formation via arginine and proline metabolism				
	K01583 K01584 K01585	Arginine decarboxylase [EC 4.1.1.19]	Arginine→agmatine	Not found
	K02626			
	K01476	Arginase [EC 3.5.3.1]	Arginine→ornithine	Not found
	K01480	Agmatinase [EC 3.5.3.11]	Arginine→putrescine	Not found
	K00797	Spermidine synthase [EC 2.5.1.16]	Putrescine→spermidine→spermine	Not found
	K01581	Ornithine decarboxylase [EC 4.1.1.17]	Ornithine→putrescine	Not found
Histidine metabolism	K01590	Histidine decarboxylase [EC 4.1.1.22]	Histidine→histamine	Not found
Lysine degradation	K01582	Lysine decarboxylase [EC 4.1.1.18]	Cadaverine production	Not found
	K23385	D-ornithine/D-lysine decarboxylase [EC 4.1.1.116]	Cadaverine production	Not found
Tyrosine metabolism	K22329 K22330	Tyrosine decarboxylase [EC 4.1.1.25]	Tyrosine→tyramine	Not found
Tryptophan metabolism	K01593	Tryptophan decarboxylase [EC 4.1.1.28]	Tryptophan→tryptamine	Not found

Detection of Antimicrobial Resistance Genes (AMR)

When determining whether or not probiotic strains are safe to use, the phenotypic characteristics of bacteria related to AMR are given significant consideration. A genomic study of probiotic bacteria could provide the information on AMR genes and their possible transferability **(EFSA, 2012).** Our previous studies on phenotypic investigations of AMR indicated that strain BBS13 was susceptible to various antibiotics **(Botthoulath *et al*., 2024).** Strain BBS13, which aims to be

utilized as a probiotic in *Nor Mai Som* or related fermented products, is required for an assessment of possible risks of AMR to validate the safety issues by genotypic analysis. The distribution of AMR genes in strain BBS13 was analyzed bioinformatically based on three databases: ResFinder 4.1, RAST through the KEGG pathways, and CARD (RGI 6.0.1). **Table 5** showed that using ResFinder 4.1's default parameters (90% threshold and 60% minimum length), no AMR genes were discovered in the genome of BBS13. However, the RAST analysis of the KEGG pathways revealed beta-lactam resistance, 31.93% identity was observed in strain BBS13 for vancomycin resistance (*vanY* gene in *vanB* cluster). The same pattern was found by **Kwon *et al*. (2021),** wherein no AMR genes were identified in the genomes of strain *L. plantarum* Q180 and *L. plantarum* DSM 20174T under the ResFinder databases. Also, **Chokesajjawatee *et al*. (2020)** investigated the absence of hits for AMR genes in the *L. plantarum* BCC9546 genome using the search functionality of ResFinder. Resistance to other beta-lactam drugs cannot be ruled out before additional research has been conducted. This is due to the fact that beta-lactamase is a broad enzyme family with variations in their substrate specificity **(Philippon *et al*., 2016; Chokesajjawatee *et al*., 2020).** Moreover, the majority of LAB-carried intrinsic resistance or natural resistance to some antibiotics, particularly *Lactobacillus* spp. that are resistant to vancomycin have D-lactate or D-serine residue rather than D-alanine residue, which often prevents vancomycin binding. This resistance is not transmissible and chromosomally encoded **(Fraqueza, 2015; Yushchuk *et al*., 2020; Selim, 2022).** EFSA's technical recommendations have established that *L. plantarum* strains do not require vancomycin breakpoints **(EFSA, 2012b).** The limited repertoire of AMR genes contained in the databases can lead to the failure in detecting the AMR at the default, high-stringency setting. As a result, ResFinder, CARD, and RAST linked KEGG databases mostly concentrate on the AMR determinants of pathogenic bacteria, the AMR genes of non-pathogenic bacteria, such as those from LAB, notably *Lactobacillus*, are typically rarely listed. Because of this, it is important to be aware of the challenges of searching for AMR genes using the most recent version of these databases for non-pathogenic bacteria. Probiotic strains were studied at the species level during the safety evaluation, and *L. plantarum* strains, which are extensively used as probiotics, were frequently declared to be safe. However, it has been noted in numerous studies that safety concerns like AMR, virulence, and the capacity to produce toxins differ depending on the strain even within the same species. It is recommended that the safety assessment of new probiotic candidate strains be performed at the strain level **(Cebeci and Gürakan, 2003; Oliveira *et al*., 2022).** As a result of this study, it can be assumed that strain BBS13 do not pose a threat to human health because of their antibiotic resistance.

Table 5 List of antimicrobial resistance genes analyzed by Rapid Annotation using Subsystems Technology (RAST) linked to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and by Comprehensive Antibiotic Resistance Database (CARD, RGI 5.0.0 tool in *Lactiplantibacillus plantarum* BBS13

AMR genes detection				
Not found any AMR genes *L. plantarum* BBS13 under ResFinder4.1 tool				
Under RAST through the KEGG pathways				
***L. plantarum* BBS13**				
Resistance	**Kegg ID**	**Gene name**	**Product/function**	**Output**
beta-lactam resistance	K17836	*penP* beta-lactamase class A [EC 3.5.2.6]	Penicillin-binding proteins (PBPs)	Found
Under CARD, RGI 6.0.1 tool				
Resistance	**AMR gene family**	**Drug class**	**Resistance Mechanism**	**% Identity of matching region**
***L. plantarum* BBS13**				
Vancomycin resistance (*vanY* gene in *vanB* cluster)	*vanY*, glycopeptide resistance gene cluster	Glycopeptide antibiotic	Antibiotic target alteration	31.93%

Bacterial Mobile Genetic Element (MGEs)

The primary cause for concern in regard to AMR/VF, or toxin genes found in beneficial non-pathogenic bacteria is the risk that these genes will be transferred to other potentially pathogenic bacteria. This transfer could result in problems and a reduction in the treatment's overall efficacy. Mobile genetic elements (plasmids and bacteriophages), which are the most likely carriers in the intercellular genetic exchange through transformation/conjugation and the transduction processes **(Li *et al*., 2018).** Thus, strain BBS13 should be evaluated for gene transfer risks to ensure that it poses no safety concerns and to utilize this strain as a probiotic in *Nor Mai Som* or related fermented products.

L. plantarum BBS13 was analyzed for plasmids using *oriT*finder tool, and the results shown none of this strains' plasmids had any *oriT* of a conjugative plasmid or a chromosome-borne integrative and conjugative element, which suggests that self-transmission will not proceed via conjugative transfer. This is consistent with the findings of **Chokesajjawatee *et al*. (2020),** who reported that no *OriT* was predicted and no bacteriophage genes in *L. plantarum* BCC9546 were present in its chromosome and concluded that the AMR genes present pose no risk of transfer to other bacteria. **Kwon *et al*. (2021)** proposed that the prophage sequences discovered in the genome of strain *L. plantarum* Q180 may not be inducible and is secure from transferability of the AMR/VF or toxin genes by a prophage, and, even if *L. plantarum* DSM 20174^{T} was present in these genes, it may have minimal transferability of AMR/VF or toxin genes via MGEs. Therefore, the conclusion that strain BBS13 does not pose safety risk in terms of the functional and transferable of AMR/VF, or toxin genes was put forth. The above findings offered the potential of using this strain as a health-promoting starter culture in culinary products.

CONCLUSION

Several studies have used WGS to characterize LAB genome for various purposes and in the present study, through the above method, the selected strain from the Lao fermented bamboo shoots was identified *L. plantarum* BBS13. The genomes presented various main features such as genome size, GC content, number of proteins coding sequences (CDSs), tRNA, and rRNA. The analysis by RAST tool linked to the KEGG pathway indicated that L-acetate dehydrogenase (L-LDH; EC 1.1.1.27) and D-lactate dehydrogenase (D-LDH; EC 1.1.1.28) genes were found in strain, which are responsible for lactic acid production. In addition, linamarase or β-glucosidases (EC 3.2.1.21) was also contained in its genomes. More importantly, there was absence of virulence factors, biogenic amines, or antimicrobial resistance genes in strain. Genomic information on BBS13 could guide genetic engineering techniques through genetic manipulation to enhance particular products. Most importantly, there is assurance that the above strain is safe to use as probiotic bacterium in food products.

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REFERENCES

Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., ... & McArthur, A. G. (2023). CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic acids research, 51*(D1), D690-D699. https://doi.org/10.1093/nar/gkac920

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. (Accessed: 10th April 2018).

Anjana, A., & Tiwari, S. K. (2022). Bacteriocin-producing probiotic lactic acid bacteria in controlling dysbiosis of the gut microbiota. *Frontiers in Cellular and Infection Microbiology*, 12, 851140. https://doi.org/10.3389/fcimb.2022.851140

Arai, K., Kamata, T., Uchikoba, H., Fushinobu, S., Matsuzawa, H., & Taguchi, H. (2001). Some *Lactobacillus* L-lactate dehydrogenases exhibit comparable catalytic activities for pyruvate and oxaloacetate. *Journal of bacteriology*, *183*(1), 397-400.https://doi.org/10.1128/jb.183.1.397-400.2001

Arjun, O. K., Sethi, M., Parida, D., Dash, J., Das, S. K., Prakash, T., & Senapati, S. (2024). Comprehensive physiological and genomic characterization of a potential probiotic strain, *Lactiplantibacillus plantarum* ILSF15, isolated from the gut of tribes of Odisha, India. *Gene*, *931*, 148882. https://doi.org/10.1016/j.gene.2024.148882

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., ... & Zagnitko, O. (2008). The RAST Server: rapid annotations using subsystems technology. *BMC genomics, 9*, 1-15. https://doi.org/10.1186/1471-2164-9-75
Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114-2120.https://doi.org/10.1093/bioinformatics/btu170
Botthoulath, V., Upaichit, A., & Thumarat, U. (2018). Identification and in vitro assessment of potential probiotic characteristics and antibacterial effects of *Lactobacillus plantarum subsp. plantarum* SKI19, a bacteriocinogenic strain isolated from Thai fermented pork sausage. *Journal of Food Science and Technology*, *55*, 2774-2785. https://doi.org/10.1007/s13197-018-3201-3
Botthoulath, V., Dalmacio, I. F., & Elegado, F. B. (2024). Physico-chemical and functional properties of the lao fermented bamboo shoots (Nor Mai Som) inoculated with potential probiotic bacteria, *Pediococcus pentosaceus* BBS1 and *Lactiplantibacillus plantarum* BBS13. *Food Chemistry Advances, 5,* 100803. https://doi.org/10.1016/j.focha.2024.100803]
Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., ... & Xia, F. (2015). RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific reports*, *5*(1), 1-6.https://doi.org/10.1038/srep08365
Carpi, F. M., Coman, M. M., Silvi, S., Picciolini, M., Verdenelli, M. C., & Napolioni, V. (2022). Comprehensive pan-genome analysis of *Lactiplantibacillus plantarum* complete genomes. *Journal of Applied Microbiology*, *132*(1), 592-604. https://doi.org/10.1111/jam.15199
Cebeci, A., & Gürakan, C. (2003). Properties of potential probiotic *Lactobacillus plantarum* strains. *Food microbiology*, *20*(5), 511-518. https://doi.org/10.1016/S0740-0020(02)00174-0
Chokesajjawatee, N., Santiyanont, P., Chantarasakha, K., Kocharin, K., Thammarongtham, C., Lertampaiporn, S., ... & Visessanguan, W. (2020). Safety assessment of a nham starter culture *Lactobacillus plantarum* BCC9546 via whole-genome analysis. *Scientific reports, 10*(1), 10241. https://doi.org/10.1038/s41598-020-66857-2
Davis, J. J., Wattam, A. R., Aziz, R. K., Brettin, T., Butler, R., Butler, R. M., ... & Stevens, R. (2020). The PATRIC Bioinformatics Resource Center: expanding data and analysis capabilities. *Nucleic acids research*, 48(D1), D606-D612.https://doi.org/10.1093/nar/gkz943
Dlamini, Z. C., Langa, R. L., Aiyegoro, O. A., & Okoh, A. I. (2019). Safety evaluation and colonisation abilities of four lactic acid bacteria as future probiotics. *Probiotics and antimicrobial proteins*, *11*(2), 397-402. https://doi.org/10.1007/s12602-018-9430-y
EFSA Panel on Biological Hazards (BIOHAZ). (2011). Scientific opinion on risk based control of biogenic amine formation in fermented foods. *Efsa Journal*, *9*(10), 2393.https://doi.org/10.2903/j.efsa.2011.2393
EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). (2012). Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal*, *10*(6), 2740. https://doi.org/10.2903/j.efsa.2012.2740
Fadahunsi, I. F., Busari, N. K., & Fadahunsi, O. S. (2020). Effect of cultural conditions on the growth and linamarase production by a local species of *Lactobacillus fermentum* isolated from cassava effluent. *Bulletin of the National Research Centre*, *44*, 1-14.https://doi.org/10.1186/s42269-020-00436-3
Fraqueza, M. J. (2015). Antibiotic resistance of lactic acid bacteria isolated from dry-fermented sausages. *International journal of food microbiology, 212*, 76-88.https://doi.org/10.1016/j.ijfoodmicro.2015.04.035
Gao, Y., Li, D., & Liu, X. (2014). Bacteriocin-producing *Lactobacillus sakei* C2 as starter culture in fermented sausages. *Food control*, *35*(1), 1-6. https://doi.org/10.1016/j.foodcont.2013.06.055
García-Cano, I., Rocha-Mendoza, D., Kosmerl, E., Zhang, L., & Jiménez-Flores, R. (2020). Technically relevant enzymes and proteins produced by LAB suitable for industrial and biological activity. *Applied Microbiology and Biotechnology*, *104*, 1401-1422. https://doi.org/10.1007/s00253-019-10322-2
Gezginç, Y., Karabekmez-erdem, T., Tatar, H. D., Ayman, S., Ganiyusufoğlu, E., & Dayısoylu, K. S. (2022). Health promoting benefits of postbiotics produced by lactic acid bacteria: Exopolysaccharide. *Biotech Studies*, *31*(2), 61-70. https://doi.org/10.38042/biotechstudies.1159166
Kwon, Y. J., Chun, B. H., Jung, H. S., Chu, J., Joung, H., Park, S. Y., ... & Jeon, C. O. (2021). Safety assessment of *Lactiplantibacillus* (formerly *Lactobacillus*) *plantarum* Q180. *Journal of Microbiology and Biotechnology*, *31*(10), 1420.https://doi.org/10.4014/jmb.2106.06066
Kwong, J. C., McCallum, N., Sintchenko, V., & Howden, B. P. (2015). Whole genome sequencing in clinical and public health microbiology. *Pathology*, 47(3), 199-210. https://doi.org/10.1097/PAT.0000000000000235
Lee, I., Chalita, M., Ha, S. M., Na, S. I., Yoon, S. H., & Chun, J. (2017). ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. *International journal of systematic and evolutionary microbiology*, *67*(6), 2053-2057. https://doi.org/10.1099/ijsem.0.001872
Lee, I., Ouk Kim, Y., Park, S. C., & Chun, J. (2016). OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *International journal of systematic and evolutionary microbiology*, 66(2), 1100-1103. https://doi.org/10.1099/ijsem.0.000760
Lei, V., Amoa-Awua, W. K. A., & Brimer, L. (1999). Degradation of cyanogenic glycosides by *Lactobacillus plantarum* strains from spontaneous cassava fermentation and other microorganisms. *International journal of food microbiology*, *53*(2-3), 169-184.https://doi.org/10.1016/S0168-1605(99)00156-7
Li, X., Xie, Y., Liu, M., Tai, C., Sun, J., Deng, Z., & Ou, H. Y. (2018). oriTfinder: a web-based tool for the identification of origin of transfers in DNA sequences of bacterial mobile genetic elements. *Nucleic acids research*, *46*(W1), W229-W234. https://doi.org/10.1093/nar/gky352
Li, Y., Wang, L., Ju, J., Yu, B., & Ma, Y. (2013). Efficient production of polymer-grade D-lactate by *Sporolactobacillus laevolacticus* DSM442 with agricultural waste cottonseed as the sole nitrogen source. *Bioresource technology*, *142*, 186-191.https://doi.org/10.1016/j.biortech.2013.04.124
Liu, B., Zheng, D., Jin, Q., Chen, L., & Yang, J. (2019). VFDB 2019: a comparative pathogenomic platform with an interactive web interface. *Nucleic acids research*, 47(D1), D687-D692. https://doi.org/10.1093/nar/gky1080
Lv, L. X., Hu, X. J., Qian, G. R., Zhang, H., Lu, H. F., Zheng, B. W., ... & Li, L. J. (2014). Administration of *Lactobacillus salivarius* LI01 or *Pediococcus pentosaceus* LI05 improves acute liver injury induced by D-galactosamine in rats. *Applied microbiology and biotechnology*, *98*, 5619-5632. https://doi.org/10.1007/s00253-014-5638-2
Ma, K., Maeda, T., You, H., & Shirai, Y. (2014). Open fermentative production of l-lactic acid with high optical purity by thermophilic *Bacillus coagulans* using excess sludge as nutrient. *Bioresource Technology*, *151*, 28-35.. https://doi.org/10.1016/j.biortech.2013.10.022
Mao, B., Yin, R., Li, X., Cui, S., Zhang, H., Zhao, J., & Chen, W. (2021). Comparative genomic analysis of *Lactiplantibacillus plantarum* isolated from different niches. *Genes, 12*(2), 241. https://doi.org/10.3390/genes12020241
Michlmayr, H., & Kneifel, W. (2014). β-Glucosidase activities of lactic acid bacteria: mechanisms, impact on fermented food and human health. *FEMS microbiology letters*, *352*(1), 1-10. https://doi.org/10.1111/1574-6968.12348
Midha, S., Ranjan, M., Sharma, V., Kumari, A., Singh, P. K., Korpole, S., & Patil, P. B. (2012). Genome sequence of *Pediococcus pentosaceus* strain IE-3. https://doi.org/10.1128/jb.00897-12
Nout, M. J. R., & Sarkar, P. K. (1999, November). Lactic acid food fermentation in tropical climates. In Lactic Acid Bacteria: Genetics, Metabolism and Applications: Proceedings of the Sixth Symposium on lactic acid bacteria: genetics, metabolism and applications, 19–23 September 1999, Veldhoven, The Netherlands (pp. 395-401). Dordrecht: *Springer Netherlands*. https://doi.org/10.1007/978-94-017-2027-4_26
Oliveira, F. S., da Silva Rodrigues, R., De Carvalho, A. F., & Nero, L. A. (2023). Genomic analyses of *Pediococcus pentosaceus* ST65ACC, a bacteriocinogenic strain isolated from artisanal raw-milk cheese. *Probiotics and Antimicrobial Proteins*, *15*(3), 630-645. https://doi.org/10.1007/s12602-021-09894-1
Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., ... & Stevens, R. (2014). The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic acids research, 42*(D1), D206-D214.https://doi.org/10.1093/nar/gkt1226
Pakbin, B., Brück, W. M., & Rossen, J. W. (2021). Virulence factors of enteric pathogenic *Escherichia coli*: A review. *International journal of molecular sciences, 22*(18), 9922. https://doi.org/10.3390/ijms22189922
Pariza, M. W., Gillies, K. O., Kraak-Ripple, S. F., Leyer, G., & Smith, A. B. (2015). Determining the safety of microbial cultures for consumption by humans and animals. *Regulatory Toxicology and Pharmacology*, *73*(1), 164-171. https://doi.org/10.1016/j.yrtph.2015.07.003
Peng, X., Ed-Dra, A., & Yue, M. (2023). Whole genome sequencing for the risk assessment of probiotic lactic acid bacteria. Critical Reviews in *Food Science and Nutrition, 63*(32), 11244-11262. https://doi.org/10.1080/10408398.2022.2087174
Philippon, A., Slama, P., Dény, P., & Labia, R. (2016). A structure-based classification of class A β-lactamases, a broadly diverse family of enzymes. *Clinical microbiology reviews*, *29*(1), 29-57. https://doi.org/10.1128/cmr.00019-15
Qureshi, N., Gu, Q., & Li, P. (2020). Whole genome sequence analysis and in vitro probiotic characteristics of a *Lactobacillus* strain *Lactobacillus paracasei* ZFM54. *Journal of Applied Microbiology*, *129*(2), 422-433. https://doi.org/10.1111/jam.14627
Richter, M., & Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proceedings of the National Academy of Sciences*, *106*(45), 19126-19131. https://doi.org/10.1073/pnas.0906412106
Salvetti, E., Orrù, L., Capozzi, V., Martina, A., Lamontanara, A., Keller, D., ... & Spano, G. (2016). Integrate genome-based assessment of safety for probiotic strains: *Bacillus coagulans* GBI-30, 6086 as a case study. *Applied microbiology and biotechnology*, *100*, 4595-4605. https://doi.org/10.1007/s00253-016-7416-9
Selim, S. (2022). Mechanisms of gram-positive vancomycin resistance. *Biomedical reports*, 16(1), 1-6.https://doi.org/10.3892/br.2021.1490
Sharma, A., Lee, S., & Park, Y. S. (2020). Molecular typing tools for identifying and characterizing lactic acid bacteria: a review. *Food science and biotechnology*, *29*, 1301-1318. https://doi.org/10.1007/s10068-020-00802-x
Shi, C., Chen, Y., Li, C., Al-Asmari, F., Cui, H., & Lin, L. (2024a). Potential Application of *Lactiplantibacillus plantarum in* Food Bio-preservation–A Comprehensive Review with a Focus on the Antibacterial and Anti-Virulence

Effects on Foodborne Pathogens. *Food Reviews International*, 1-27. https://doi.org/10.1080/87559129.2024.2317283
Shi, H., Zhao, Y., Wang, W., Zhou, Y., Liang, Y., Wu, R., & Wu, J. (2024b). The potential of lactic acid bacteria in fermented herbs-derived food products. *Food Bioscience*, 104714. https://doi.org/10.1016/j.fbio.2024.104714
Singh, N., Singh, J., & Singh, K. (2018). Small at size, big at impact: microorganisms for sustainable development. Microbial bioprospecting for sustainable development, *Springer, Singapore*,3-28. https://doi.org/10.1007/978-981-13-0053-0_1
Sun, L., Zhang, C., Lyu, P., Wang, Y., Wang, L., & Yu, B. (2016). Contributory roles of two l-lactate dehydrogenases for l-lactic acid production in thermotolerant *Bacillus coagulans*. *Scientific Reports, 6*(1), 37916. https://doi.org/10.1038/srep37916
Swetwiwathana, A., & Visessanguan, W. (2015). Potential of bacteriocin-producing lactic acid bacteria for safety improvements of traditional Thai fermented meat and human health. *Meat science*, 109, 101-105. https://doi.org/10.1016/j.meatsci.2015.05.030
Syrokou, M. K., Paramithiotis, S., Drosinos, E. H., Bosnea, L., & Mataragas, M. (2022). A comparative genomic and safety assessment of six *Lactiplantibacillus plantarum subsp. argentoratensis* strains isolated from spontaneously fermented Greek wheat sourdoughs for potential biotechnological application. *International Journal of Molecular Sciences, 23*(5), 2487. https://doi.org/10.3390/ijms23052487
Taboada, B., Ciria, R., Martinez-Guerrero, C. E., & Merino, E. (2012). ProOpDB: Pro karyotic Op eron D ata B ase. *Nucleic acids research,* 40(D1), D627-D631. https://doi.org/10.1093/nar/gkr1020
Tarrah, A., Pakroo, S., Corich, V., & Giacomini, A. (2020). Whole-genome sequence and comparative genome analysis of *Lactobacillus paracasei* DTA93, a promising probiotic lactic acid bacterium. Archives of microbiology, *202*, 1997-2003. https://doi.org/10.1007/s00203-020-01883-2
Torriani, S., Felis, G. E., & Dellaglio, F. (2001). Differentiation of *Lactobacillus plantarum*, *L. pentosus,* and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR assay with *recA* gene-derived primers. Applied and environmental microbiology, *67*(8), 3450-3454.https://doi.org/10.1128/AEM.67.8.3450-3454.2001
Vesterlund, S., Vankerckhoven, V., Saxelin, M., Goossens, H., Salminen, S., & Ouwehand, A. C. (2007). Safety assessment of *Lactobacillus* strains: presence of putative risk factors in faecal, blood and probiotic isolates. *International journal of food microbiology, 116*(3), 325-331. https://doi.org/10.1016/j.ijfoodmicro.2007.02.002
Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS computational biology*, *13*(6), e1005595. https://doi.org/10.1371/journal.pcbi.1005595
Xie, Y., Wang, Y., Han, Y., Zhang, J., Wang, S., Lu, S., ... & Jia, L. (2022). Complete genome sequence of a novel *Lactobacillus paracasei* TK1501 and its application in the biosynthesis of isoflavone aglycones. *Foods, 11*(18), 2807.https://doi.org/10.3390/foods11182807
Yang, S., Deng, C., Li, Y., Li, W., Wu, Q., Sun, Z., ... & Lin, Q. (2022). Complete genome sequence of *Lactiplantibacillus plantarum* ST, a potential probiotic strain with antibacterial properties. Journal of Animal Science and Technology, *64*(1), 183. 10.5187/jast.2022.e7
Yilmaz, B., Bangar, S. P., Echegaray, N., Suri, S., Tomasevic, I., Manuel Lorenzo, J., ... & Ozogul, F. (2022). The impacts of *Lactiplantibacillus plantarum* on the functional properties of fermented foods: A review of current knowledge. *Microorganisms, 10*(4), 826. https://doi.org/10.3390/microorganisms10040826
Yushchuk, O., Binda, E., & Marinelli, F. (2020). Glycopeptide antibiotic resistance genes: distribution and function in the producer actinomycetes. *Frontiers in Microbiology*, *11*, 1173.https://doi.org/10.3389/fmicb.2020.01173
Zankari, E., Allesøe, R., Joensen, K. G., Cavaco, L. M., Lund, O., & Aarestrup, F. M. (2017). PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *Journal of Antimicrobial Chemotherapy*, *72*(10), 2764-2768. https://doi.org/10.1093/jac/dkx217
Zhang, Z. Y., Liu, C., Zhu, Y. Z., Wei, Y. X., Tian, F., Zhao, G. P., & Guo, X. K. (2012). Safety assessment of Lactobacillus plantarum JDM1 based on the complete genome. *International Journal of Food Microbiology*, *153*(1-2), 166-170.. https://doi.org/10.1016/j.ijfoodmicro.2011.11.003
Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M., Harris, H. M., Mattarelli, P., ... & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus:* Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International journal of systematic and evolutionary microbiology, 70*(4), 2782-2858. https://doi.org/10.1099/ijsem.0.004107
Zheng, Z., Sheng, B., Ma, C., Zhang, H., Gao, C., Su, F., & Xu, P. (2012). Relative catalytic efficiency of *ldhL*-and *ldhD*-encoded products is crucial for optical purity of lactic acid produced by *Lactobacillus* strains. *Applied and environmental microbiology*, *78*(9), 3480-3483. https://doi.org/10.1128/AEM.00058-12