

# Genomic insight of two indigenous probiotics *Lactobacillus plantarum* Dad-13 and *Lactobacillus plantarum* Mut-7 from different origins of Indonesian fermented foods

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**Abstract.** Suroto DA, Hasan PN, Rahayu ES. 2021. Genomic insight of two indigenous probiotics *Lactobacillus plantarum* Dad-13 and *Lactobacillus plantarum* Mut-7 from different origins of Indonesian fermented foods. *Biodiversitas* 22: 5491-5500. *Lactobacillus plantarum* Dad-13 and *Lactobacillus plantarum* Mut-7 are probiotics isolated from traditional fermented foods. In the present study two strains of *L. plantarum* were sequenced using Illumina NovaSeq PE150 platform and in silico analysis was performed using RAST webserver to unravel the functional metabolic between two strains. The *L. plantarum* Dad-13 and Mut-7 genome sizes were 3.25 and 3.98 Mb with GC content of them being almost similar i.e. 44.4 and 44.3%, respectively. Two lactobacilli also had some differences in their metabolic genes, particularly in carbohydrate subsystem. *L. plantarum* Mut-7 had genes related to the metabolisms of plant cell wall components such as dextrin, arabinose, xylose indicated the adaptation to their environmental origins (fermented dried cassava), while those genes were not observed in *L. plantarum* Dad-13 which was isolated from fermented buffalo's milk (*dadih*). Further analysis using BAGEL4 webserver exhibited their different potential to synthesize bacteriocins, *L. plantarum* Dad-13 was predicted to produce more types of bacteriocins than *L. plantarum* Mut-7. This investigation revealed that *L. plantarum* Dad-13 and *L. plantarum* Mut-7 have a great potential related with their properties as probiotics.

**Keywords:** Bacteriocins, fermented foods, genomic analysis, *Lactobacillus plantarum*, probiotics

## INTRODUCTION

Lactic acid bacteria (LAB) are well known to inhabit diverse environments, such as in carbohydrate-rich food of plants (Sakandar et al. 2019; Ashaolu and Reale 2020) and animal origin (Albayrak and Duran 2021; Gupta et al. 2021), mucosa and intestinal of human or animal (Medjaoui et al. 2016; Fečkaninová et al. 2019; Li et al. 2020), terrestrial and aquatic environment (Sica et al. 2010; Fhoula et al. 2013; Prasirtsak et al. 2013).

LAB have a substantial role in fermented foods, such that they enhance flavor, increase nutritional value and even play a role as food preservatives (Nuraida 2015; Behera et al. 2020). LAB are known to have probiotic properties due to their health beneficial effects (Linares et al. 2017; Ayivi et al. 2020). Probiotics can maintain the condition of the digestive tract by inhibiting the growth of pathogenic bacteria and increasing favorable bacteria (Sarkono et al. 2010; Bermudez-britho et al. 2012; Trush et al. 2020). The functional properties of probiotic are strain dependent, and each strain has unique characteristics and functional properties (Shiroda and Manning 2020; Sophatha et al. 2020).

*Lactobacillus plantarum* plays an important role in well-known fermented foods of animal and vegetable origin including kimchi, sauerkraut, cheese, kefir,

sourdough, vegetable pickles (Nuraida 2015; Tamang et al. 2016) and recognized as Generally Recognized as Safe (GRAS) by Food and Drug Administration (Behera et al. 2018). *L. plantarum* showed survival ability against gastric acid and bile salt and can colonize in the human intestinal tract (Le and Yang 2018; Zhang et al. 2020) make them a suitable candidate for probiotics development. In addition, *L. plantarum* is attracting medical science due to its cholesterol-lowering properties, reducing blood level, anticancer properties, kidney protection properties, and immunomodulatory properties (Arasu et al. 2016).

LAB as probiotics are also strongly affected by their ability to produce bacteriocins as antibacterial substances that can inhibit the growth of pathogenic bacteria (Bermudez-britho et al. 2012; Ramu et al. 2015). *L. plantarum* produces a group of bacteriocins known as plantaricins that show antibacterial properties against a handful of pathogenic bacteria (Todorov et al. 2016; Butorac et al. 2020; Ahaddin et al. 2021), and show potential for food preservatives (Kareem and Razavi 2019).

Indonesian indigenous fermented foods have a long history of benefiting from lactic acid bacteria. *L. plantarum* has been isolated from several kinds of Indonesian indigenous fermented food, such as tempoyak, mandai, gatot, growol, dadih, bekasam etc (Nuraida 2015). Several strains of *L. plantarum* have been isolated from plant and

animal origin of fermented foods. *L. plantarum* Dad-13 isolated from dadih (fermented buffalo's milk) and *L. plantarum* Mut-7 isolated from gatot (fermented dried cassava) (Rahayu 2003). These isolates showed health benefit properties and potential candidates for local probiotics. *L. plantarum* Dad-13 could increase the population of *Lactobacilli* and inhibit the growth of *Enterobacteriaceae* (Rahayu et al. 2016); *L. plantarum* Mut-7 could increase the population of *Lactobacillus* in fecal and could survive in intestine (Fitrianingthias et al. 2018). The safety assessment of two *L. plantarum* also has been studied (Rahayu et al. 2019; Ikhsani et al. 2020). The whole study indicated that those strains are promising local probiotics for Indonesian population.

The aim of the present study was to perform genomic analysis of two *L. plantarum* strains to explore their strain-specific characteristic and their capability to produce secondary metabolites, especially bacteriocins.

## MATERIAL AND METHODS

### Bacterial strains and culture

*L. plantarum* Dad-13 and Mut-7 were obtained from FNCC (Food and Nutrition Culture Collection), Center of Food and Nutrition Studies Universitas Gadjah Mada, Yogyakarta, Indonesia. Isolates were inoculated in de Man Rogosa and Sharpe (MRS) broth and incubated at 37°C for 36-48 hours.

### Bacterial genomic extraction

Genomic DNA was extracted by the SDS method (Lim et al. 2016a). The harvested DNA was detected by agarose gel electrophoresis and quantified by Qubit® 2.0 Fluorometer (Thermo Scientific).

### Library construction and sequencing

A total amount of 1µg DNA per sample was used as input material for DNA sample preparation. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, DNA sample was fragmented by sonication to a size of 350bp, then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing for further PCR amplification. At last, PCR products were purified (AMPure XP system) and libraries were analysed for size distribution by Agilent2100 Bioanalyzer and quantified using real-time PCR. The genome sequencing was performed using Illumina NovaSeq PE150 platform.

### Genome assembly

De novo assembly of raw reads data was performed by SOAP denovo software, followed by SPAdes software (version SPAdes-3.12.0-Linux) in a Linux environment (Bankevich et al. 2012), different K-mers (99 and 127) were selected for assembly. According to the project type,

assembly result was obtained with the optimal K-mer and the least scaffolds. The results from SPAdes were assembled using AbySS software (Simpson et al. 2009). K-mer 64 was selected for assembly. The assembly results of three softwares were integrated with CISA software (Lin and Liao 2013), and assembly result with the least scaffolds were selected. The SOAPGapcloser software (Luo et al. 2021) was used to fill the gap of preliminary assembly results.

### Bioinformatic analysis

Genome annotation was identified by using the online program Rapid Annotation using Subsystem Technologies (RAST) SEED (<http://rast.nmpdr.org/>) (Overbeek et al. 2014). Secondary metabolites gene clusters were predicted using AntiSMASH 6 (<https://antismash.secondarymetabolites.org/>) (Blin et al. 2021). Further annotations of bacteriocin gene clusters were performed using Bagel4 (<http://bagel4.molgenrug.nl/index.php>) (van Heel et al. 2014).

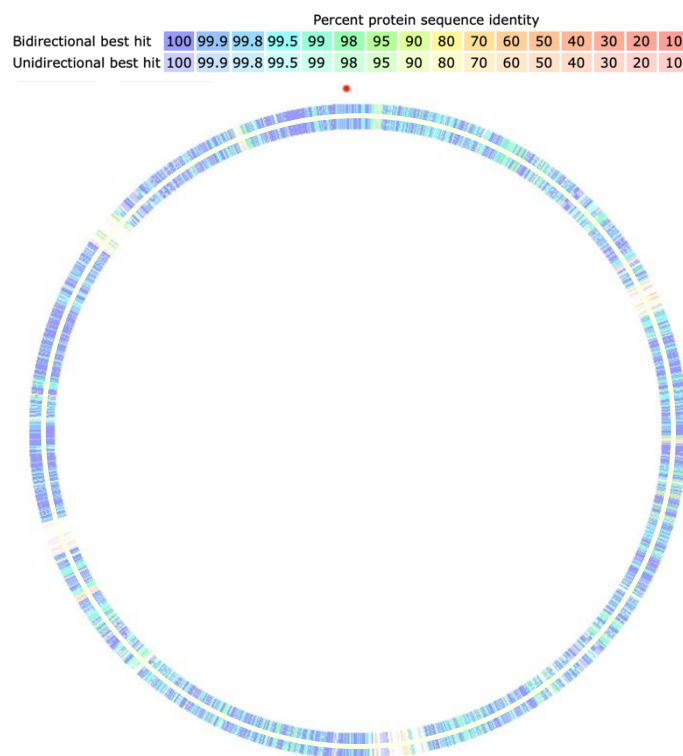
## RESULTS AND DISCUSSION

### Genome features of *Lactobacillus plantarum* Dad-13 and *L. plantarum* Mut-7

The present study revealed that two indigenous strains have different genome sizes. The circular *L. plantarum* Mut-7 genome was approximately 0.147 Mb larger than *L. plantarum* Dad-13, with GC content being almost similar i.e. 44.4 and 44.3 %. Consequently, Mut-7 contained more coding sequences as well as RNA. Gene prediction and annotation showed that *L. plantarum* Dad-13 and Mut-7 possess 3074 and 3214 coding sequences, respectively (Table 1). The genome size and GC content of Mut-7 and Dad-13 are within the range observed for most *L. plantarum* strains (Yu et al. 2017; Kim et al. 2020). The different size and number of genes have also been studied in *L. plantarum* (Yu et al. 2017; Rodrigo-torres et al. 2019; Kim et al. 2020). *L. plantarum* Dad-13 and Mut-7 were almost identical in the protein sequence as shown in Figure 1. However, some regions showed low identity, mainly hypothetical proteins and phages.

**Table 1.** Genome features of *Lactobacillus plantarum* Dad-13 and *L. plantarum* Mut-7

Features	<i>L. plantarum</i> Dad-13	<i>L. plantarum</i> Mut-7
Genome size (bp)	3,250,375	3,398,000
%GC content	44.4%	44.3%
RNA	75	87
Coding sequences	3074	3214
N50 (bp)	332,562	163,062
No of contigs	27	36



**Figure 1.** Graphical genomic comparison map of *Lactobacillus plantarum* strains with Seed Viewer sequenced-based comparison tool in RAST webserver. From outer to inner ring: strain Mut-7, Dad-13. Colors denote amino acid similarity percentage to the reference genome, from purple (100%) to light red (10%)

### In silico analysis of functional metabolic pathways

Based on RAST analysis, the coverage subsystems of *L. plantarum* Dad-13 had a total of 1273 features, with 1217 non-hypothetical subsystem and 56 hypothetical subsystem. *L. plantarum* Mut-7 had 1337 features including 1279 nonhypothetical subsystems and 58 hypothetical subsystems. In both strains genes related to carbohydrate subsystem category had high proportion followed by amino acid and derivatives, protein metabolisms, cell wall and capsule, cofactors, vitamins and prosthetic group, and RNA metabolisms (Figure 2). Mut-7 encoded 456 genes on carbohydrate subsystem, while Dad-13 encoded only 416 genes, Mut-7 encoded 231 genes on amino acids and derivatives followed by 223 genes in Dad-13. The number of genes related to capsule and cell wall, DNA metabolism, phage and prophages, and amino acid derivative protein metabolisms in *L. plantarum* Mut-7 were 147, 98, 28, 231, 188, respectively, which was comparatively larger than *Lactobacillus* Dad-13. Plasmids, which may transfer their genetic material to other microorganisms, were not detected in both lactobacilli.

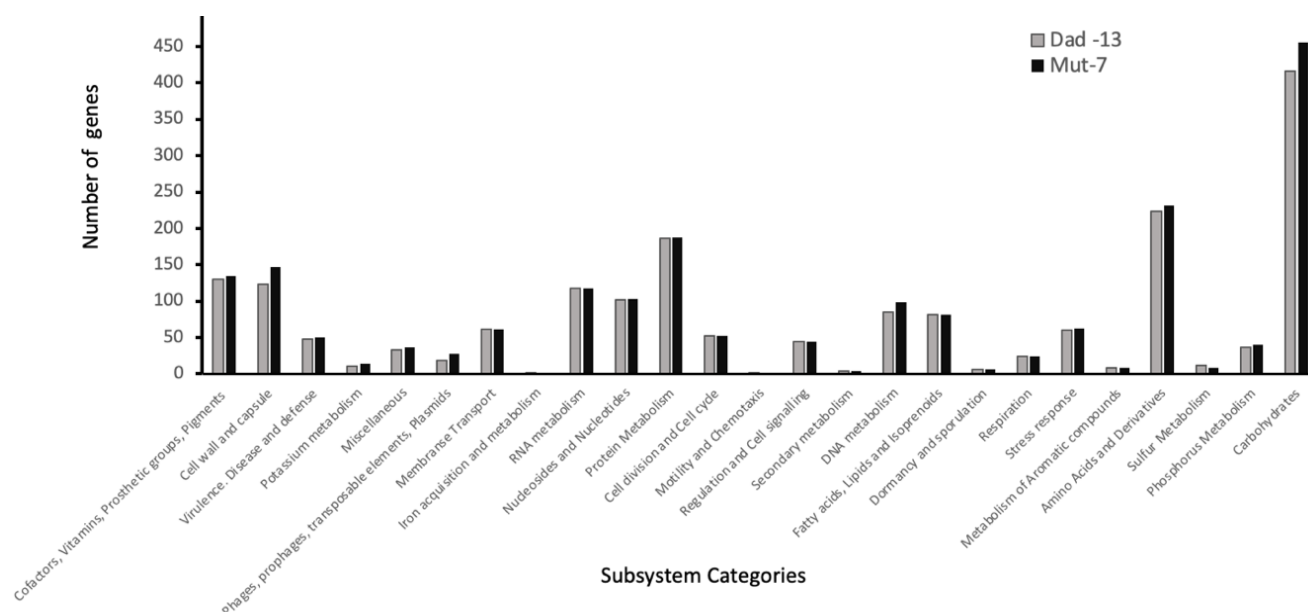
The two strains have mostly common functional genes, however there were some differences in both lactobacilli. Especially in carbohydrate subsystem category, followed by cell wall and capsule subsystem, DNA metabolism, phages, prophages, transposable elements, and amino acid and derivatives category (Figure 2). While the numbers of other genes were almost similar, but some genes were present in several copies, or some unique genes were present in only one strain. *L. plantarum* Mut-7 and *L.*

*plantarum* Dad-13 had 56 and 13 extra unique genes, respectively (Table 2). In *L. plantarum* Mut-7, 17 of extra unique genes were related to carbohydrate metabolism, especially fructose-1,6-bisphosphatase, PEP Pyruvate carboxyl transferase, maltose-6'-phosphate glucosidase, some gene related to xylose, arabinose utilization subsystem. Indeed, they also shared some genes related to carbohydrate metabolism such as for maltodextrin utilization, but maltose-6'-phosphate glucosidase was absent in Dad-13. Exclusively several genes for xylose and arabinose utilization were only present in Mut-7. The unique genes for monosaccharide utilization seem related to plant-based origin of *L. plantarum* Mut-7 in which sugars like dextrin, arabinose, and xylose are common in the plant product. This phenomenon was also observed in *Lactococcus lactis* from plant origin that show the presence of dextrin, arabinose, and xylose while the one from animal origin did not, indicated the adaptation to their growth in plant cell wall substances (Siezen et al. 2008; Xiao et al. 2021).

*Lactobacillus plantarum* Dad-13 possessed chitinase, a beta glucosidase related to lichenan, a complex glucan consisting of glucosyl connected with  $\beta$ 1,3 and  $\beta$ 1,4 bonds, and sucrose 6 phosphate hydrolase. It also occurred in betaglucosidase metabolism and in fructooligoscharides utilization, in which they both shared 39 genes. Even though Mut-7 shared some genes related to chitin and N acetyl glucosamine, but the chitinases were only present in Dad-13.

**Table 2.** Features of unique genes in *Lactobacillus plantarum* Dad-13 and *L. plantarum* Mut-7

Functional subsystems	<i>Lactobacillus plantarum</i> Dad-13		<i>Lactobacillus plantarum</i> Mut-7	
	No of unique genes	Description	No of unique genes	Description
Carbohydrate	4	Chitinase, beta-Glucoside metabolism, transcriptional antiterminator of lichenan operon, sucrose-6-phosphate hydrolase	17	Fructose-1,6-bisphosphatase pyruvate carboxyl transferase, maltose-6'-phosphate glucosidase galactitol operon regulator (Transcriptional antiterminator), arabinose-proton symporter, L-arabinose isomerase, ribulokinase, transcriptional repressor of arabinoside utilization operon, L-ribulose-5-phosphate 4-epimerase, alpha-xylosidase, xylose isomerase, xylose-responsive transcription regulator: ROK family
Capsule and cell wall	-	-	7	Capsular and extracellular polysaccharide rhamnose containing glycan synthesis, exopolysaccharide (Glycosyl transferase, group 2 family protein), teichoic and lipoteichoic acids biosynthesis CDP glycerol:poly(glycerophosphate) glycerophosphotransferase (EC 2.7.8.12)
DNA metabolism	3	Exonuclease family protein YhaO, bacterial DNA-cytosine methyltransferase bacterial MutL-MutS system, MutS domain protein, family 4	11	RecT, DNA topoisomerase III, Type I restriction-modification system, DNA-methyltransferase subunit M, Type I restriction-modification system, restriction subunit R, Type I restriction-modification system, specificity subunit S CRISPR-associated protein Cas1, CRISPR-associated protein Cas2, CRISPR-associated protein, Csn1 family
Amino acids and derivative	-	-	3	D-serine dehydratase transcriptional activator, Intermediate for synthesis of Tryptophan, PAPA antibiotics, PABA, 3-hydroxyanthranilate, cysteine biosynthesis, para-aminobenzoate synthase, amidotransferase component (EC 2.6.1.85),
Phages, prophages, transposable element, plasmid	3	Phage capsid scaffolding protein, phage tail fiber protein, phage minor capsid protein,	9	Phage capsid protein, phage head maturation protease, phage DNA packaging, phage terminase small subunit, phage tail fiber proteins, phage tail proteins, phage major tail protein, phage tail assembly
Cofactor, vitamins, prosthetic group	-	-	3	NAD and NADP cofactor biosynthesis, riboflavin, FMN, and FAD metabolism
Nucleosides and Nucleotides	-	-	2	Uridine phosphorylase (EC 2.4.2.3)
Phosphorus metabolism	-	-	1	High affinity phosphate transporter and control of PHO
Regulation and cell signalling	-	-	3	Autolysis histidine kinase LytS regulation and Cell signaling, autolysis response regulator LytR, cAMP signaling in bacteria
Sulfur metabolism	3	Organic sulfur assimilation: alkanesulfonate assimilation ABC-type nitrate/sulfonate/bicarbonate transport systems, periplasmic components, Alkanesulfonates ABC transporter ATP-binding protein,	-	



**Figure 2.** Results of RAST analysis of *Lactobacillus plantarum* Dad-13 and *L. plantarum* Mut-7

*Lactobacillus plantarum* Mut-7 also had a high proportion of unique genes related to cell wall and capsule, DNA metabolism and phages, prophages, transposable elements, plasmids. In the cell wall and capsule category, genes related to capsular and extracellular polysaccharide, especially in rhamnose containing glycan synthesis and exopolysaccharide (EPS) biosynthesis were lacking in Dad-13. But, EPS biosynthesis which was encoded by glycosyl transferase group 2 family's gene was present in Mut-7, indicating a potential ability of Mut-7 to produce EPS. EPS are loosely associated with the microbial cell surface and released into the surrounding environment (Chapot-Chartier and Kulakauskas 2014). The presence of exopolysaccharide- synthesized genes is indicative of its important role in industrial applications, as they give the desired texture to products and some health benefits (Silva et al. 2019).

Genes in rhamnose containing glycan synthesis may play an important role for cell wall synthesis, in which rhamnose is one of the major sugars present in the cell wall of several bacteria *Lactococcus*, *Streptococcus* and *Enterococcus*, and *Lactobacillus* (Vinogradov 2016; Garcia-Vello et al. 2020).

*Lactobacillus plantarum* Mut-7 had a unique genes in DNA metabolism which was not present in Dad-13, most genes related to restricted modification system and Clustered Regularly Interspaced Palindromic Repeats (CRISPR) associated proteins. The presence of CRISPR-Cas proteins in Mut-7 may inhibit the conjugation and bacteriophage infection (Richter et al 2012). They can also prevent the natural transformation of foreign nucleic acids fragments, which can contribute to making this strain genetically more stable (He et al. 2018; Tarrah et al. 2020). These sequences have been shown to be involved in resistance to bacteriophages (Deem et al. 2020). They play a critical role in the adaptation and persistence of a

microbial host in a particular ecosystem (Westra et al.2020).

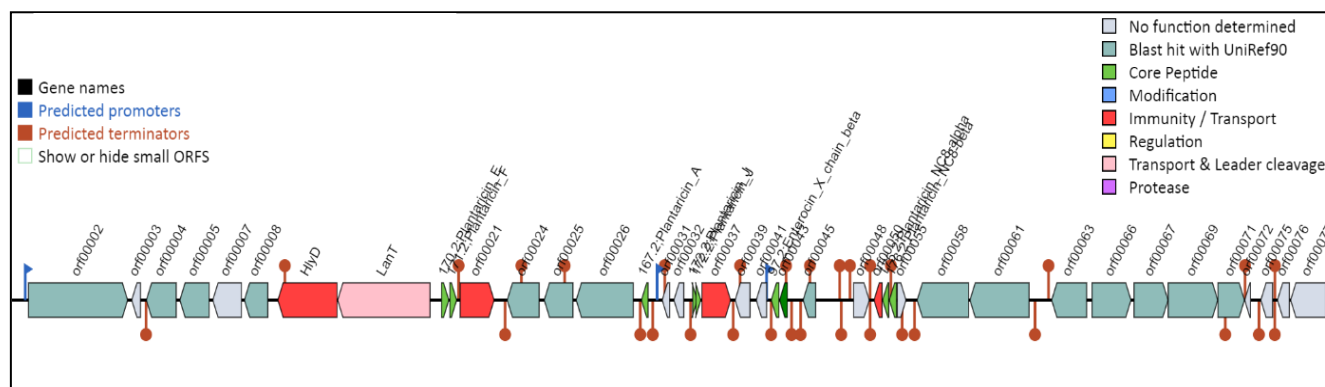
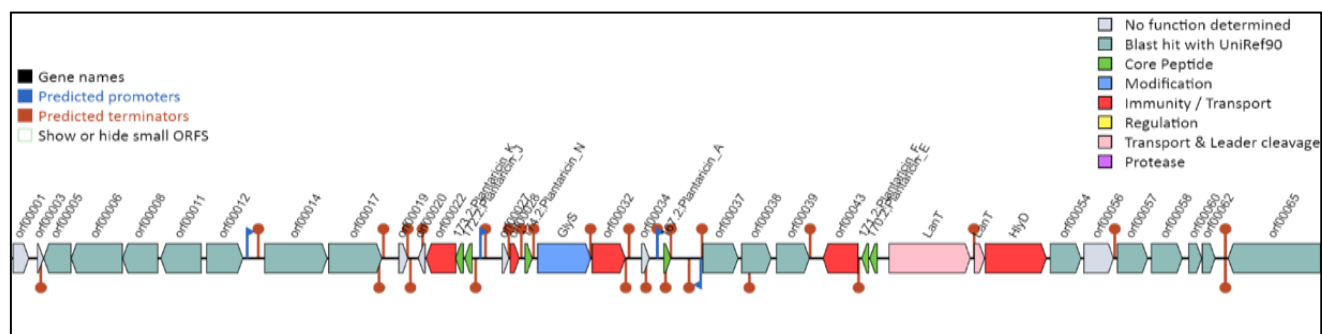
Unlike *L. plantarum* Mut-7, *L. plantarum* Dad-13 lacked genes encoding phage capsid proteins, phage head maturation protease, phage DNA packaging, phage packaging machinery, phage terminase small subunit, phage tail fiber proteins, phage major tail protein, phage tail proteins, phage tail assembly. However, Dad-13 possessed genes encoding phage capsid scaffolding protein, phage minor capsid protein, phage tail fiber protein which were absent in Mut-7. In addition, *L. plantarum* Dad-13 also possessed a unique gene in sulfur metabolism category. Minor number of unique genes in *L. plantarum* Mut-7 was also observed in amino acid derivatives, cofactors, vitamins, prosthetic group, nucleosides and nucleotides, phosphorus metabolism regulation and cell signalling subsystem category.

### In silico analysis of genes related to secondary metabolism

The results exhibited that both strains had four clusters related to the secondary metabolism, namely terpene, Polyketide Synthase (PKS) type III, Ribosomally Synthesized and Post Translationally Modified Peptide (RiPP), and cyclic lactone autoinducer. Each cluster was predicted to have almost similar size, except RiPP cluster (Table 3). Terpene biosynthetic gene cluster is predicted to involve in phytoene biosynthesis which belongs to carotenoid group. PKS type III showed high homology to chalcone synthetase. encode the same function as hydroxymethylglutaryl-CoA synthase. Chalcone synthase is an enzyme that is responsible for the biosynthesis of flavonoid compounds (Waki et al. 2020), and is widely present in plants, fungi, and bacteria (Austin and Noel 2003; Lim et al. 2016b).

**Table 3.** Predicted secondary metabolite gene clusters in *Lactobacillus plantarum* Dad-13 and *L. plantarum* Mut-7

Biosynthetic gene clusters	<i>L. plantarum</i> Dad-13	<i>L. plantarum</i> Mut-7
	Region/size	Region/size
Terpene	58,162 nt-79,043 nt/ 20,881nt	434,387 nt-455,268 nt/ 20,881
PKS Type III	309,144 nt-350,313 nt/ 41,169 nt	129,364 nt-170,533 nt/ 41,169 nt
Cyclic lactone	197,434 nt-218,139 nt/ 20,705 nt	31,932 nt-52,687 nt/ 20,755 nt
Ribosomally Synthesized and Post Translationally Modified Peptide (RiPP)	441,720 nt-414,644 nt/ 27,076 nt	48,957 nt-60,786 nt/ 11,829 nt

**Figure 3.** Area of Interest (AOI) *Lactobacillus plantarum* DAD-13 in scaffold 2. Genes with function are determined from left to right (gene name function). *orf1-6* hypothetical protein; *orf8*, galactoside O-acetyltransferase; *orf9*, drug efflux pump; *orf11*, S-amino-6(5-phospho-D-ribitylamino)uracil phosphatase; *orf14*, HTH-type transcriptional activator; *orf15*, sugar phosphatase; *orf17*, branched chain amino acid transport system carrier protein; *orf20*, Na(+)/H(+) antiporter; *orf22*, hypothetical protein; *comC*, plantaricin NC8β; *comC*, plantaricin NC8α; *orf28*, putative immunity protein; *orf31*, transposase; *orf33*, Na(+)/H(+) -K(+) antiporter; *orf35*, lactococin; *comC*, enterocin X chain beta; *orf37-39*, hypothetical protein; *orf41*, putative bacteriocin immunity protein; *plantaricin J*, Plantaricin J; *plantaricin J*, plantaricin J; *orf46-47*, hypothetical protein; *plantaricin A*, plantaricin A; *orf52*, bacteriocin production related histidine kinase; *orf53*, response regulator PlnC; *orf54*, response regulator PlnD; *orf59*, immunity protein plnI; *plantaricin F*, plantaricin F; *plantaricin E*, plantaricin E; *lanT*, bacteriocin ABC transporter; *hylD*, accessory factor for ABC-transporter PlnH; *orf70*, protein Yvdc; *orf71*, CPBP intramembrane metalloprotease; *orf73*, PlnS; *orf74*, PLnS; *orf75*, hypothetical protein; *orf77*, DNA helicase IV**Figure 4.** Area of Interest (AOI) *Lactobacillus plantarum* Mut-7 in scaffold 5. Genes with function are determined from left to right (gene name function). *orf1*, hypothetical protein; *orf3*, hypothetical protein; *orf5*, galactoside O-acetyltransferase; *orf6*, methylenomycin A resistance; *orf8*, 5-amino-6 (5-phospho D-ribitylamino) uracil phosphate; *orf11*, HTH transcriptional activator; *orf12*, sugar phosphatase; *orf14*, branched-chain amino acid system; *orf17*, Na(+)/H(+) antiporter; *orf19*, hypothetical protein; *orf20*, plnR; *orf22*, putative bacteriocin immunity protein plnM; *comC*, plantaricin K; *plantaricin J*, plantaricin J; *orf27*, hypothetical protein; *orf28*, LACPL immunity protein PlnM; *plantaricin N*, plantaricin N; *glyS*, PlnO; *orf32*, LACPL immunity protein PlnI; *orf34*, hypothetical protein; *plantaricin A*, plantaricin A; *orf37*, Bacteriocin related histidine kinase; *orf38*, response regulator PlnC; *orf39*, response regulator PlnD; *orf43*, LACPL immunity protein PlnI; *plantaricin F*, plantaricin F; *plantaricin E*, plantaricin E; *lanT*, bacteriocin ABC-transporter ATP-binding and permease protein; *lanT*, bacteriocin ABC-transporter ATP-binding and permease protein; *HylD*, Accessory factor for ABC-transporter PlnH; *orf54*, PlnS; *orf56*, CPBP family inter membran metalloprotein; *orf57*, PLnS; *orf59*, PlnS; *orf60*, toxin HigB; *orf62*, plnY; *orf65*, DNA Helicase IV

Since Ribosomally Synthesized and Post Translationally Modified Peptide (RiPP) cluster is closely associated with bacteriocins (Mathur et al. 2021). Scaffold 2 of *L. plantarum* Dad-13 and scaffold 5 of *L. plantarum* Mut-7 which harbour RiPP cluster were genome mined using BAGEL4 to know whether by both strains may produce bacteriocins. *Lactobacillus*, known to produce bacteriocins (Gaspar et al. 2018; Wang et al. 2018; Mira-Villalobos et al. 2020) and other secondary metabolites such as L13 (3-phenyl-1, 2, 4-benzotriazine), 3-phenyllactic acid (Arasu et al. 2013; Sentürk et al. 2020). *L. plantarum* is well known to produce plantaricins, a bacteriocin showing various bactericidal and bacteriostatic activities (Zhu et al. 2014; Wen et al. 2016; Zhao et al. 2016; Wang et al. 2018). Plantaricins together with Pediocins belong to a class II bacteriocins, with small molecular weight of less than 10 kDa, heat-stable, and non-lantibiotic peptides (Barbosa et al. 2021).

It was observed that each cluster can potentially produce several bacteriocins types. The size of bacteriocins gene clusters in Dad-13 and Mut-7 were 30.591 nt and 29.498 nt, respectively as predicted by BAGEL4. *L. plantarum* Dad-13 predicted to produce eight types of bacteriocins, namely plantaricins A, E, F, J, NC8 alpha, NC8 beta, lactococin, and enterocin (Figure 3, Table 4), whereas Mut-7 was predicted to produce six types of bacteriocins : plantaricin A, E, F, J, K, and N (Figure 4, Table 5). Each of bacteriocin cluster was equipped with HylD and Lant T genes which were involved in transport and leader peptide cleavage. In Dad-13, their position was in the upstream region of plantaricin encoding genes, whereas in Mut 7 it was in the downstream region of the bacteriocin gene clusters. LanT gene homologues encoded

the bacteriocin ABC transporter, the ATP binding protein, and the permease protein PlnG.

Various types of plantaricins produced by *L. plantarum* have antibacterial properties and their mechanism for bacterial growth inhibition varies considerably (Kareem and Razavi 2019). However, at present the prospects of plantaricins are numerous such as, biopreservation agents of raw food, antibacterial packaging agent, anti-cancer, antioxidants, and anti cholesterol agent (Syaputri and Iwahashi 2020). Dad-13 was predicted to produce not only plantaricin bacteriocin but also lactococin and enterocin. Mut-7 was predicted to produce only plantaricin type bacteriocin, particularly plantaricin K/N, in addition to A, E, F, J but not plantaricin NC, lactococin, and enterocin. Plantaricin, E/F and J/K showed antibacterial against *Staphylococcus epidermidis* by destruction of cell membrane and also show synergistic effect with several antibiotics (Selegård et al. 2019). Plantaricin NC8  $\alpha/\beta$  was found active against *Staphylococcus* spp. and possibly to be developed as an adjuvant in combination therapy to potentiate the effects of antibiotics and reduce their overall (Bengtsson 2020). The plantaricin A showed a narrow spectrum and weak bacteriocins, acting as inducer for production of plantaricin E/F and J/K (Kareem and Ravazi 2019).

The difference of bacteriocins or plantaricins produced by Dad-13 and Mut-7 may affect their antimicrobial properties. *L. plantarum* Dad-13 was able to inhibit the growth of *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella typhi*, while *L. plantarum* Mut-7 showed similar activity but not inhibited *S. typhi* (Rahayu et al. 2015).

**Table 4.** BAGEL4 webserver's prediction of plantaricins and their amino acid sequences produced *Lactobacillus plantarum* Dad-13

Type of bacteriocins	Amino acid sequences	Match score
Plantaricin E	MLQFEKLQYSRLPQKKLAKISGGFNRRGGYNFGKSVRHVVDAIGSVAGIRGILKSIR	100%
Plantaricin F	MKKFLVLRDRELNAISGGVFHAYSARGVRNNYKSAVGPADWVISAVRGFIHG	100%
Plantaricin A	VIIMKIQIKSMKQLSNKEMQKIVGGKSSAYSLSQMGATAIKQVKKLFKKWGW	97.92%
Plantaricin J	LEGSWKNFWSFKFYAGEAGRVVRMSLNTCLNRSHAL	66.67%
Plantaricin J	MDKTLKNLDTVDFAFASISNNKLNGVVGRLLKFLV	74.04%
Lactococin	VIKMKNNNFQALQKNELSKVKGGSNNKFWTWAGYTYENWRISRRAFNLRQRKNTMTHH	75.86%
Enterococin X chain	MRKSISNFKALNEKELGAVNGGIWQWIVGGLGFLAGDAWSHSDQISSGIKKRKKKGYG	53.91%
Plantaricin NC8 alpha	MDKFEKISTSNLEKISGGDLTTKLWSSWGYLGGKKARWNLKHPYVQF	100%
Plantaricin NC8 beta	MNNLNKFSTLGKSSLSQIEGGSVPTSVYTLGILKLSAYKHKRTIEKSFNKGIFYH	100%

**Table 5.** BAGEL4 webserver's prediction of plantaricins and their amino acid sequences produced by *Lactobacillus plantarum* Mut-7

Type of bacteriocins	Amino acid sequence	Match score
Plantaricin K	MKIKLTVLNEFEELTADAENKISGGRRSRKNGIGYAIQYAFGAVERAVLGGSRDYNK	100%
Plantaricin J	MTVNMKIKDLVDVDAFAPISNNKLNGVVGGGAWKNFWSSLRKGFYDGEAGRAIRR	100%
Plantaricin N	MKSLDKIAGLGIEMAEKDLTTVEGGKNYSKTWWYKSLTLLGKVAEGTSSAWHGLG	100%
Plantaricin A	VIIMKIQIKGMKQLSNKEMQKIVGGKSSAYSLSQMGATAIKQVKKLFKKWGW	100%
Plantaricin F	MKKFLVLRDRELNAISGGVFHAYSARGVRNNYKSAVGPADWVISAVRGFIHG	100%
Plantaricin E	MLQFEKLQYSRLPQKKLAKISGGFNRRGGYNFGKSVRHVVDAIGSVAGIRGILKSIR	98.21%

The results of present study highlight the singularity of strains and their metabolic capacities. Both strains were similar in many ways, but some differences were found exclusively in their carbohydrate metabolism, capsule and cell wall synthesis, DNA metabolism, prophage/phage, and bacteriocins production. *L. plantarum* Mut-7 originated from gatot (fermented dried cassava) possesses a larger genome size than *L. plantarum* Dad-13 originated from dadih (fermented buffalo's milk). Mut-7 also possesses unique genes for plant carbohydrates metabolism indicating the adaptation to the plant environment. Therefore, Dad-13 produces more types of bacteriocins than Mut-7, which may affect their antibacterial properties.

Our approach to sequence *L. plantarum* from different origins of natural habitat provides quick insight into their diversity. It is essential to isolate and sequence other lactic bacteria from various origins of indigenous fermented foods to untap their metabolic or probiotics potential. Thus, their beneficial properties can be applied to improve food processing, food safety, and human health.

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