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Characterization of genomic, physiological, and probiotic features *Lactiplantibacillus plantarum* DY46 strain isolated from traditional lactic acid fermented shalgam beverage

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ABSTRACT

Lactiplantibacillus plantarum is a significant probiotic where it could be found in ubiquitous niches. In this study, a new *Lb. plantarum* strain DY46 was isolated from a traditional lactic-acid-fermented beverage called shalgam. The whole genome of the DY46 was sequenced and obtained sequences were assembled into a 3.32 Mb draft genome using PATRIC (3.6.8.). The DY46 genome consists of a single circular chromosome of 3,332,827 bp that is predicted to carry 3219 genes, including 61 tRNA genes, 2 rRNA operons. The genome has a GC content of 44.3% includes 98 predicted pseudogenes, 25 complete or partial transposases and 3 intact prophages. The genes encoding enzymes related in the intact EMP (Embden–Meyerhof–Parnas) and PK (phosphoketolase) pathways were predicted using BlastKOALA which is an indicator of having facultative heterofermentative pathways. DY46 genome also predicted to carry genes of *Pln E*, *Pln F* and *Pln K* showing the antimicrobial potential of this bacterium which can be linked to *in vitro* antagonism tests that DY46 can inhibit *S.enterica* sv. *Typhimurium* ATCC14028, *K. pneumonie* ATCC13883, and *P. vulgaris* ATCC8427. Moreover, it is determined that all resistome found in its genome were intrinsically originated and the strain was found to be tolerant to acid and bile concentrations by mimicking human gastrointestinal conditions. In conclusion, *L. plantarum* DY46 is a promising bacterium that appears to have certain probiotic properties, confirmed by "*in vitro*" and "*in silico*" analyses, and is a potential dietary supplement candidate that may provide functional benefits to the host.

1. Introduction

Lactic acid bacteria (LAB) by definition are gram (+), catalase (−), anaerobic but aerotolerant fermentative. Lactic acid bacteria are utilized in the food biotech industry and possess well-documented beneficial effects on health (Buron-Moles, Chailyan, Dolejs, Forster, & Mikš, 2019; Evanovich, De Souza Mendonça Mattos, & Guerreiro, 2019 2019). The LAB are considered as GRAS (Generally Recognized as Safe) by USDA and QPS (Qualified Presumption of Safety) by EFSA (Seddik et al., 2017). They usually act as bioprotective cultures owing to the biosynthesis of antimicrobials such as bacteriocin, nisin, enterocin, curvaticin, helveticin, plantaricin etc. (Gaggia, Di Gioia, Baffoni, & Biavati, 2011; Jyoti P.; Tamang, Shin, Jung, & Chae, 2016) which exist in many fermented dairy and vegetable products (Jiang et al., 2012; Jyoti Prakash Tamang, Tamang, Schillinger, Guigas, & Holzapfel, 2009; Mokoena, 2017; Ribeiro, Stanton, Yang, Ross, & Silva, 2018; Wouters, Grosu-Tudor, Zamfir, & De Vuyst, 2013). The *Lactobacillus* genus is a major and comprehensive group among the LAB. Lactobacilli have been isolated from various ecological environments and different studies have revealed differences in their physiological and genetic levels (Seddik et al., 2017). Certain *Lactobacillus* species, such as *Lactobacillus rhamnosus* and *Lactobacillus delbrueckii*, could only be found in limited

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ecological niches, contrarily *Lactiplantibacillus plantarum* strains are found in widespread niches such as meat, dairy products, vegetables, wine, silage, gastrointestinal, urogenital and vaginal tracts (Fidanza, Panigrahi, & Kollmann, 2021). This omnipresent trait is the hallmark of the marvellous capacities of metabolic pathway varieties and evolutionary adaptation (Fiocco et al., 2010). Furthermore, *Lb. plantarum* play a crucial role in manufacturing fermented vegetables, beverages, kefir, cheese, fermented meat products by improving the nutritional value, flavour, food preservation of fermented foods (Behera, El Sheikha, Hammami, & Kumar, 2020; El Sheikha & Hu, 2020). Recently, *Lb. plantarum* is highly studied by many research groups around the world, due to its possible positive effects on health and its antibacterial potential on food safety and biopreservation technology (Russo et al., 2017). Besides, several strains of *Lb. plantarum* are known to have probiotic features and these have been used for the development of potential live oral vaccines and therapeutic and functional foods (Parente et al., 2010).

The Shalgam is a highly popular and traditional lactic-acid-fermented beverage in Turkey's west and southeast regions, where it is mostly produced and consumed (Ekinci et al., 2016; Tanguler & Erten, 2012). It is characterized by red colour, cloudy appearance and sour-soft taste which is derived from black-carrot (*Daucus carota* var. L.) used as the main ingredient in shalgam production (Erten, Tanguler, & Canbaş, 2008). It has been produced at both industrial and home-scale levels. Albeit, there is no standard application of production methodologies in the industry. It could be declared that two different methods are used in shalgam production which are traditional and direct methods (Fig. 1.). The traditional method consists of sourdough fermentation and carrot fermentation. Conversely, in the direct method, the sourdough fermentation step is taken out and only carrot fermentation is implemented (Agirman, Settanni, & Erten, 2021; Tanguler & Erten, 2012).

Shalgam microbiota is reported to carry LAB thus showing presumptive health benefits against several diseases and health risks (Ekinci et al., 2016; Seddik et al., 2017; Tanguler, Cankaya, Agcam, & Uslu, 2021). Only a few studies attempted to uncover the microbial composition of the shalgam microbiome, for example, according to Agirman

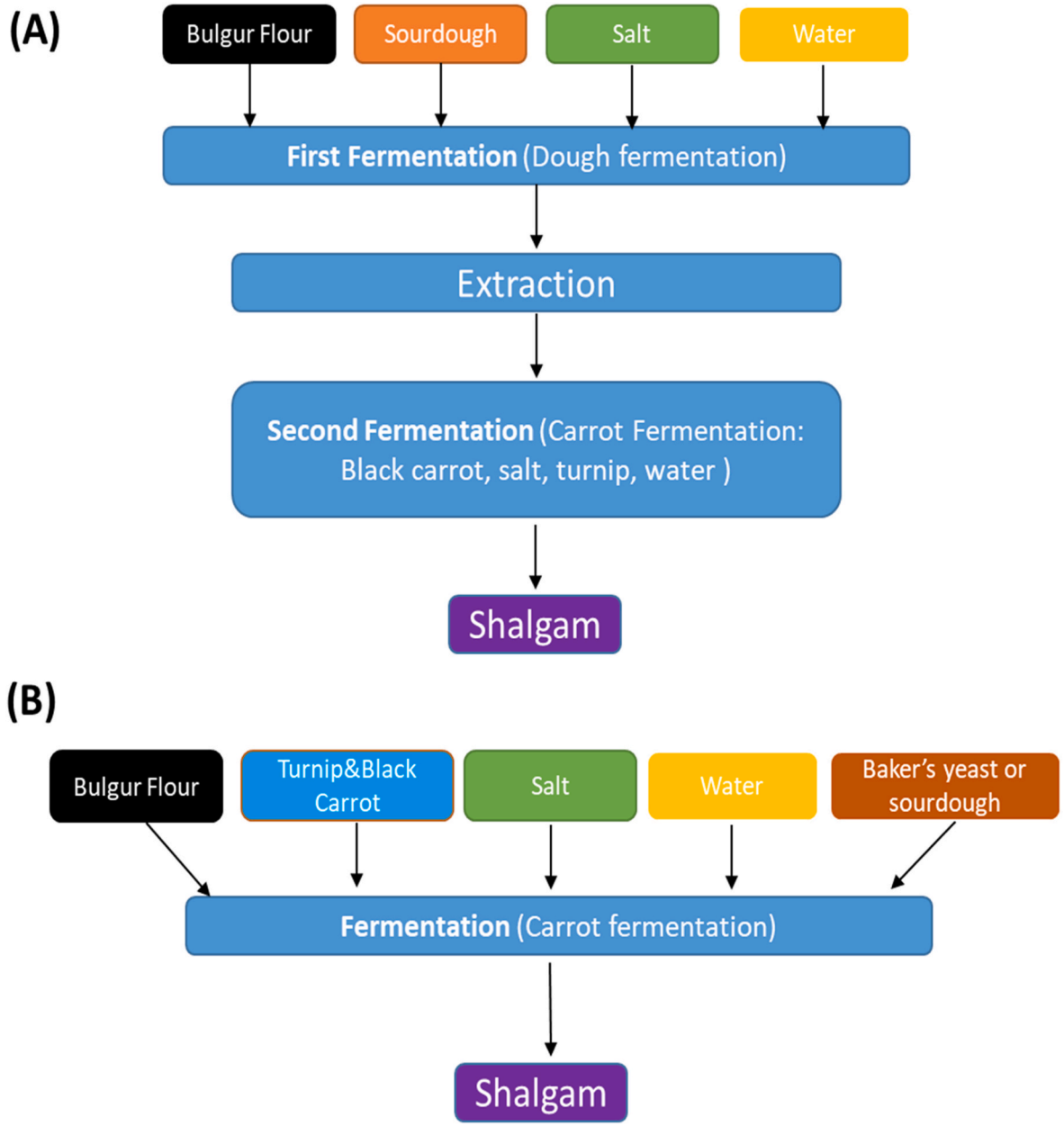

Fig. 1. Shalgam production methodologies. A) traditional method, B) direct method (Erten et al., 2008).

et al. (2021) shalgam is a rich source of *Lactobacilli* species where *Lactocaseibacillus paracasei* and *Lactiplantibacillus plantarum* are predominant strains in Shalgam microbial composition. In another study performed by Tanguler and Erten (2012), *Lactiplantibacillus plantarum* was reported as the most dominant Lactobacilli during all fermentation steps in shalgam. Also, they stated that *Lactocaseibacillus paracasei* sp. *paracasei* is the quantitatively prevalent LAB strain after *Lb. plantarum*.

Strains of *Lb. plantarum* is well described for its probiotic functions because of its natural habitation in various environmental niches, such as all types of fermented foods, human and animal gastrointestinal tract (Zhang et al., 2012) and a number of its strains are heavily being utilized in commercial probiotic culture products. However, as of March 29, 2021, 215 genomes are belonging to *Lb. plantarum* strains were deposited to NCBI and only three of these strains were isolated from fermented plant materials (i.e. pickled cabbage, pickles, Sichuan pickle) (https://www.ncbi.nlm.nih.gov/genome/browse/#!/prokaryotes/1108/). This limits the understanding of microbial community dynamics and microbial interactions in plant-based fermented foods microbiome.

The objectives of this study were to evaluate the genomic, physiological, and probiotic characteristics of the *Lb. plantarum* DY46 strain which is isolated from Shalgam. As far as we are concerned, there is no study evaluating the probiotic characteristics of LAB isolated from shalgam, although some research was carried out on uncovering its microbiota. The strain's phenotypic features were investigated by screening and selection criteria of probiotics. On the other hand, the whole genome of the *Lb. plantarum* DY46 using Next-Generation sequencing and "*in silico*" metabolic potentials using bioinformatic tools have also been explored. This is the first report describing phenotypic and genotypic characteristics in addition to *in vitro* probiotic potentials of a novel *Lb. plantarum* DY46 strain isolated from a plant-based traditional fermented beverage "Shalgam".

2. Materials and methods

2.1. Isolation of bacterial strain and growth conditions

Lactiplantibacillus plantarum DY46 strain was isolated from a Turkish fermented shalgam juice (pH: 3.01) that was purchased from a local producer (Doktorum Yılmaz) in Adana, Turkey. A 10 mL of the shalgam sample was diluted with 90 mL of Maximum Recovery Diluent (Merck, GmbH, Darmstadt, Germany) in a Schott bottle and homogenized for 1 min with a highspeed vortex (MS-3 Basic, IKA-Werke GmbH, Staufen, Germany). Serial decimal dilutions were prepared from the same diluent and 100 μL of each diluent was spread on MRS agar (Merck). The plates were incubated at 30°C for five days in an anaerobic atmosphere. The DY46 isolate was selected from the dilution of 10^{-4} and subjected to colony purification twice. Then, gram staining and catalase tests were applied to the pure isolate of *Lb. plantarum* DY46, respectively. The cryo stocks of DY46 was prepared in MRS broth (Merck) with 25% glycerol and were stored at −80°C.

2.2. Genomic DNA extraction, identification, whole-genome sequencing and de novo assembly

Prior to DNA extraction, *Lb. plantarum* DY46 cryo culture was subcultured twice in MRS broth (Merck) followed by incubation anaerobically at 30 °C for 24h. A 1 mL fresh culture was pipetted onto a sterile eppendorf tube and centrifuged at 6000×*g* for 10 min at 4 °C. Total DNA extraction from pellet was carried out using the PureLink Genomic DNA Mini Kit (Invitrogen, Thermo-Fisher Scientific, Carlsbad, CA, USA) per manufacturer's instructions for gram-positive bacteria. The quality and concentration of genomic DNA were checked by a Qubit 3.0 fluorometer (Invitrogen, Thermo-Fisher Scientific) and agarose gel (1.5 %). Identification of the test strain was carried out by full-length nucleotide sequencing of the 16S rDNA gene. Universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were employed (Ni, Wang, Li, Cai, & Pang, 2015). Amplification reactions were prepared with a total volume of 30 μL containing, 3 units of EasyTaq DNA polymerase (TransGen Biotech, Beijing, China), 3 μL of 10x EasyTaq Buffer, 20 μM of forward primer, 20 μM reverse primer, 0.9 μL DMSO (3%), 2.4 μL 2.5 mM dNTP, 22.1 μL nuclease-free water and 50 ng template DNA. Reaction mixes were amplified with a thermal cycler (ABI Veriti 96, Thermo-Fisher Scientific) under the following conditions: the initial denaturation of DNA for 5 min at 94 °C was followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 20s, extension at 72 °C for 90s, and followed by a 5 min final extension at 72 °C. The PCR products were run on agarose gel (1.5 %) and sequenced by the Sanger method (Ficus Biotechnology, Ankara, Turkey). The obtained sequences were searched against the Basic Local Alignment Search Tool (NCBI-BLAST). After confirming the DY46 strain via 16S rDNA, the whole genome sequencing libraries were constructed using Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) and sequencing was fulfilled by Illumina Miseq platform as paired-end (PE) 2x250 bases read. The low-quality reads were filtered and assembled in the genome assembly service of PATRIC 3.6.8 (https://patricbrc.org/app/Assembly2) with an auto strategy (Davis et al., 2020).

2.3. Bioinformatic analyses

Genome annotation and comprehensive genome analysis were performed by using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and PATRIC 3.6.8. platform (Davis et al., 2020; Tatusova et al., 2016). A circular genome map of the strain genome was generated with CG view server (Stothard & Wishart, 2005). The calculation of orthologous average nucleotide identity values (OrthoANI) of the DY46 and other compared *Lb.plantarum, Lb.pentosus* and *Lb. paraplantarum* strains were implemented by OrthoANI tool v0.93.1 (Lee, Kim, Park, & Chun, 2016). Prediction of metabolic pathways of *Lb. plantarum* DY46 was carried out using BlastKOALA for scanning against the KEGG database (Kanehisa, Sato, & Morishima, 2016). Hierarchical clustering of DY46 and 70 different *Lb. plantarum* genomes were conducted in regard to KEGG pathways (EC) via JGI's IMG/M (v. 6.0) genome clustering service (Chen et al., 2021).

The bacteriocin production responsible gene cluster prediction was fulfilled using the BAGEL 4 webserver (http://bagel4.molgenrug.nl/) (Heel, Jong, Montalba, Kok, & Kuipers, 2013). Following, each member of predicted gene clusters was confirmed via the NCBI protein BLAST suite (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Theoretical isoelectric point (pI) and molecular mass (MW) of the predicted peptides were calculated by Expasy Compute pI/MW online tool (https://www.expasy.org/resources/compute-pI-mw). The prophage regions on the genome of DY46 were identified and annotated with the PHASTER-Phage Search Tool Enhanced Release (Arndt et al., 2016). In order to identify the genes transferred horizontally, all protein-coding genes obtained from PHASTER were screened against the non-redundant protein (NR) database by performing protein-BLAST. If a gene's homologous protein was found to match a microorganism other than *Lb. plantarum* ≥ 80%, that gene was considered horizontally transferred (Liu et al., 2015). A resistome screening was executed by scanning the complete genome sequences of the DY46 strain versus the ResFinder 4.1, CARD, PATRIC 3.6.8 and KEGG databases, respectively (Alcock et al., 2020; Bortolaia et al., 2020; Davis et al., 2020; Kanehisa et al., 2016). As with the phage elements, horizontal gene transfer screening was performed within the detected resistome. The complete genome sequence of *Lb. plantarum* DY46 was submitted to NCBI under accession number PRJNA697983.

2.4. Carbohydrate fermentation

The carbohydrate fermentation patterns of the DY46 strain were determined using an API 50 CHL kit (BioMérieux, Marcy l'Etoile,

France) consisting of 49 different carbohydrate tests in accordance with the manufacturer's protocols.

2.5. Determination of antibiotic susceptibility

Antibiogram assays were conducted to find out the resistance or sensitivity of the DY46 strain against commonly used antibiotics. Ready to use commercial antibiotic disks [methicillin, vancomycin, amikacin, kanamycin, azithromycin, tetracycline, penicillin G (Bioanalyse, Yenimahalle, Ankara, Turkey); ampicillin, oxacillin, carbenicillin, amoxicillin, streptomycin, erythromycin, rifampicin (Oxoid, Basingstoke, Hampshire, UK)] were employed for antibiotic susceptibility testing of *Lb. plantarum* DY46. The application of disk diffusion assay was performed according to a modified Kirby-Bauer method (Tenover, 2009). Interpretation of inhibition zone (mm) results was carried out with regard to Clinical and Laboratory Standards Institute's performance standards for antimicrobial testing (CLSI M100-S22, 2012). Results with an inhibition zone less than or equal to 14 mm were noted resistant (R). Additionally, inhibition zones greater than 20 mm were considered sensitive (S) and between 15 and 19 mm were accepted as semi-sensitive or intermediate (I).

2.6. Probiotic properties

To determine probiotic properties of the DY46: β-haemolysis, cell surface hydrophobicity, cellular auto-aggregation tests and antibacterial activity assay against several pathogens were performed, respectively. On the other hand, the growth kinetics of the DY46 were analyzed at different pH and bile concentrations. Evaluation of the β-haemolytic activity of the DY46 was performed using 5% sheep blood containing Columbia agar plate. The isolate was streaked on the Columbia agar followed by incubation at 37°C for 48 h under anaerobic conditions (Angmo, Kumari, Savitri, & Bhalla, 2016). Cell surface hydrophobicity and auto-aggregation assays were performed in compliance with Goel, Halami, and Tamang (2020). Antibacterial activity assay was executed by the agar well diffusion method (Mishra & Prasad, 2005). The supernatant of 18–20 h grown DY46 was analyzed against *Escherichia coli* O157:H7 (ATCC 43895), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 33019), *Salmonella enterica* serovar *Typhimurium* (ATCC 14028), *Proteus vulgaris* (ATCC 8427), and *Klebsiella pneumoniae* (ATCC 13883). Growth characteristics of the DY46 were investigated at different pH and bile concentrations. First, the overnight grown fresh culture of DY46 was prepared in MRS broth at 30°C. Second, the MRS medium was prepared to create media with different conditions. Third, Oxyrase (Sigma-Aldrich, USA) was added to the prepared media to reduce the oxygen level. The pH of the MRS medium was adjusted to a value between 6.8 and 8.4 which is optimum for oxyrase. After adjusting the pH value, oxyrase was added to the medium in proportions according to McMahon, Bowen, Green, Domek, and Oberg (2020). MRS medium with five different pH values (pH 2, 3, 4, 5, 7) was prepared using 3N HCl and 3N NaOH. For the preparation of bile concentrations of a different pre-separated MRS medium, the pH was adjusted to 6.5. Four different bile concentrations (0.3%, 0.5%, 1% and control (no bile)) were prepared using ox bile extract (Sigma, Germany). After preparing all media, they were incubated at 36.5°C for 30 min to activate oxyrase. Growth measurements were performed in HIDEX Sense Microplate Reader (Hidex, Finland) using 96 well-plates with lid. Each well was inoculated with 200 μl from media containing the previously indicated ratios (McMahon et al., 2020). Samples were inoculated in quadruplicate. Spectrophotometric measurements were carried out at 30°C and 37°C with a 300 rpm orbital shake. An optical density (OD) measurement at a wavelength of 600 nm was performed every 20 min at 72 h post-inoculation.

3. Results and discussion

3.1. The genome of the DY46 strain

The whole genome of the *Lb. plantarum* DY46 strain composed of a circular chromosome (Fig. 2.) of 3,332,827 bp with a GC ratio of 44.3395%, a total of 3.219 genes, comprising 3.054 protein-coding sequences, 61 tRNAs, 2 rRNAs, 4 non-coding RNAs and 98 pseudogenes (Table 1). As a result of the KEGG orthology screening, the encoded proteins identified on the genome of the DY46 strain were classified in 20 different functional categories and summarized in Table 2.

The Ortho ANI values of the DY46 and other *Lb. plantarum* strains were displayed in Fig. 3. Based on Ortho ANI results, *Lb. plantarum* DY46 showed a high level of identical genetic reciprocal similarity of 99.36%, 99.05%, 99.03%, 99.02% for *Lb. plantarum* ATCC 8014, WCFS1, ATCC 14917 and ST-III respectively. ATCC 8014 and 14917 were isolated from pickled cabbage, while the well-known probiotic strain WCFS1 was isolated from human saliva (Kleerebezem et al., 2003; Siezen et al., 2010). The strains of ATCC 8014, WCFS1 and ATCC 14917 are commercial strains and considered probiotics (Gaudana, Dhanani, & Bagchi, 2010; Liu et al., 2015). The ST-III strain was originated from a Korean fermented vegetable called "kimchi"(Wang et al., 2011). On the other side, the strains RI-113 (98.99%), Y44(98.97%) and LL441(98.7%) showed the lowest genetic similarity to the DY46 among the studied *Lb. plantarum* strains. As expected, LL441 (cheese) and RI-113 (salami) showed a greater genetic distance than other strains due to their isolation sources (Flórez & Mayo, 2018; Gonzalez, Arca, Mayo, & Suarez, 1994; Inglin & Meile, 2020). Moreover, as regards the hierarchical clustering results which are based on KEGG pathways (EC), the DY46 strain showed the highest similarity to the *Lb. plantarum* Nizo 2801 that was originated from a pickled turnip (Fig. S1).

Lb. plantarum can be found in many different environments and shares its ecological niche with *Lb. pentosus* and *Lb. paraplantarum* and other facultative heterofermentative members of the genus *Lactobacillus* (Stiles & Holzapfel, 1997). Besides, *Lb. plantarum*, *Lb. pentosus* and *Lb. paraplantarum* display very close phenotypes and are genotypically similar due to their rRNA having as same sequence identity (>99%). Therefore, these species cannot be discriminated from each other using 16S rDNA sequence analysis (Parente et al., 2010). According to Ortho ANI results which were shown in Fig. 2. *Lb. paraplantarum* and *Lb. pentosus* exhibit 85.89% and 79.93% similarity with the DY46 strain, respectively. It is usually reported that the ANI value should be above 95–96% to consider that the genomes of the two species are the same (Lee et al., 2016). This confirms that the DY46 strain belongs to the *Lb. plantarum* species.

3.2. Carbohydrate fermentation

The lactobacilli can derive ATP from heterofermentative and/or homofermentative carbohydrate fermentation based on each species/strain preference of sugar utilization. Uncovering carbohydrate fermentation patterns has been widely applied to determine phenotypic traits. Per API 50 CHL test results DY46 can metabolize 22 different carbohydrates out of 49 being tested (Table 3). It is important to note that DY46 cannot metabolize D-Sorbitol, although its genome has sorbitol-6-phosphate 2-dehydrogenase [*srlD* (K00068); EC:1.1.1.140] and the glucitol/sorbitol phosphotransferase system [*srlB* (K02781), *srlE* (K02782) and *srl A* (K02783)] genes. This might be due to the lack of expression of the abovementioned genes encoding specific enzymes required to metabolize sorbitol (Buron-Moles et al., 2019).

Lactiplantibacillus plantarum is a protean and resilient species that can grow on a wide range of carbohydrate sources. This phenotypic character is associated with genes involved in carbohydrate metabolism and transport. Most of the transporters involved in carbohydrate metabolism are located in the phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) (Gänzle & Follador, 2012; Gao et al., 2020).

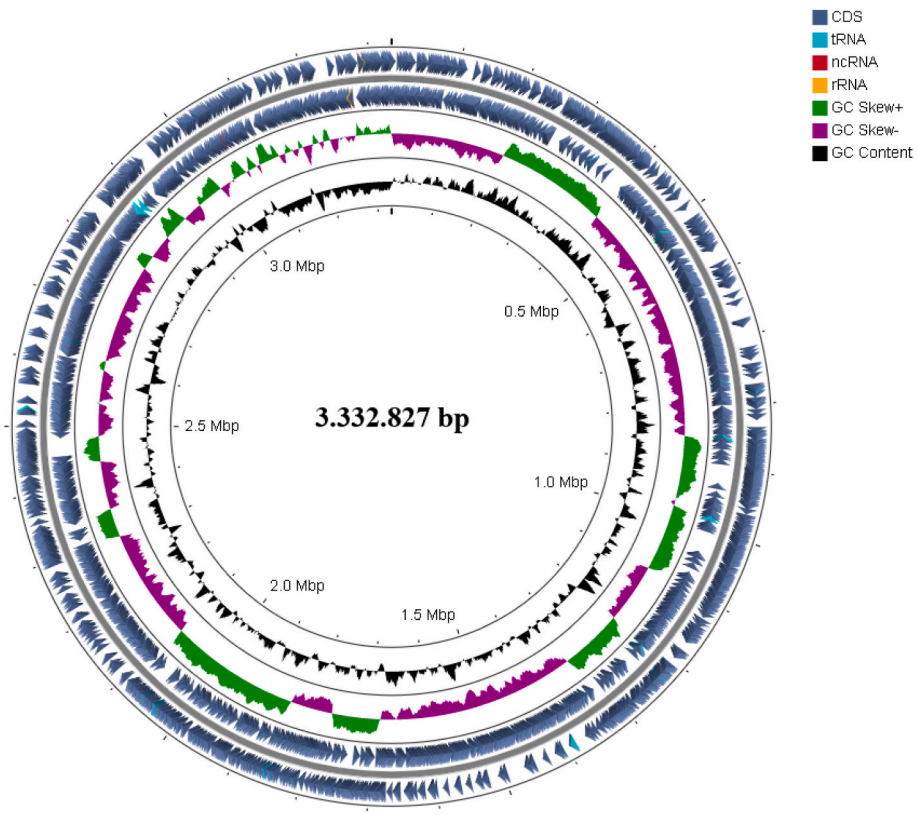

Fig. 2. The genome atlas of *Lactiplantibacillus plantarum* DY46. The 1st and 2nd outer circles illustrate prognosticated protein-coding sequences, tRNA, ncRNA, rRNA on the forward and reverse strands, respectively. The third circle depicts the GC skew (G–C)/(G + C). The fourth circle display the GC content of the genome. The last circle shows the genome size.

Table 1
Genomic features of *Lactiplantibacillus plantarum* DY46.

Item	Complete Genome
Size (bp)	3.332.827
GC content (%)	44.3395
Genes (total)	3.219
Protein coding sequences	3.054
tRNA	61
rRNA	2
Non-coding RNA	4
Pseudogenes	98

The entire PTS of DY46 strain that was encoded by its genome, comprises PTS System Enzyme I (general enzyme gene, *ptsI*, K08483), phosphocarrier protein HPr gene (*ptsH*, K02784), 26 complete/incomplete substrate-specific enzyme II (EII) complexes genes (Supplemental Table S22).

In the genome of the DY46 strain, the genes of glucose-glucoside, fructose-mannose-sorbose, glucitol, Lactose-N, N′-diacetylchitobiose-β-glucosides and N-Acetylglucoseamine EII complex families were observed as multiple copies, while L-ascorbate, sorbose, mannitol and galactitol EII complex genes were found as single copies. Additionally, the DY46 possesses several other carbohydrate transporter encoding genes on its genome. However, their substrate specificity is unknown and could not be predicted. According to Kleerebezem et al. (2003) various sugar transporter systems are known to import more than one substrate. *Lb. plantarum* is classified into the facultative heterofermentative *Lactobacillus* species, which utilize the sugars by way of glycolysis [Embden-Meyerhof-Parnas (EMP) pathway] or the phosphoketolase (PK) pathway, leading to homolactic or heterolactic fermentation routes, respectively (Liu et al., 2015).

DY46 genome carries 6-phosphofructokinase 1 [EC:2.7.1.11],

Table 2
KEGG orthology (KO) categories of identified protein-coding sequences in the genome of *Lactiplantibacillus plantarum* DY46.

KO Number	Functional category	Gene Number	Proportion (%)
09101	Carbohydrate metabolism	203	12.78
09102	Energy metabolism	33	2.08
09103	Lipid metabolism	40	2.52
09104	Nucleotide metabolism	72	4.53
09105	Amino acid metabolism	99	6.23
09106	Metabolism of other amino acids	18	1.13
09107	Glycan biosynthesis and metabolism	20	1.26
09108	Metabolism of cofactors and vitamins	69	4.34
09110	Metabolism of terpenoids and polyketides	13	0.82
09111	Xenobiotics biodegradation and metabolism	9	0.57
09120	Genetic information processing	164	10.32
09130	Environmental information processing	156	9.82
09140	Cellular processes	19	1.20
09150	Organismal systems	6	0.38
09181	Protein families: metabolism	41	2.58
09182	Protein families: genetic information processing	236	14.85
09183	Protein families: signalling and cellular processes	178	11.20
09191	Unclassified: metabolism	112	7.05
09192	Unclassified: genetic information processing	66	4.15
09193	Unclassified: signaling and cellular processes	32	2.01

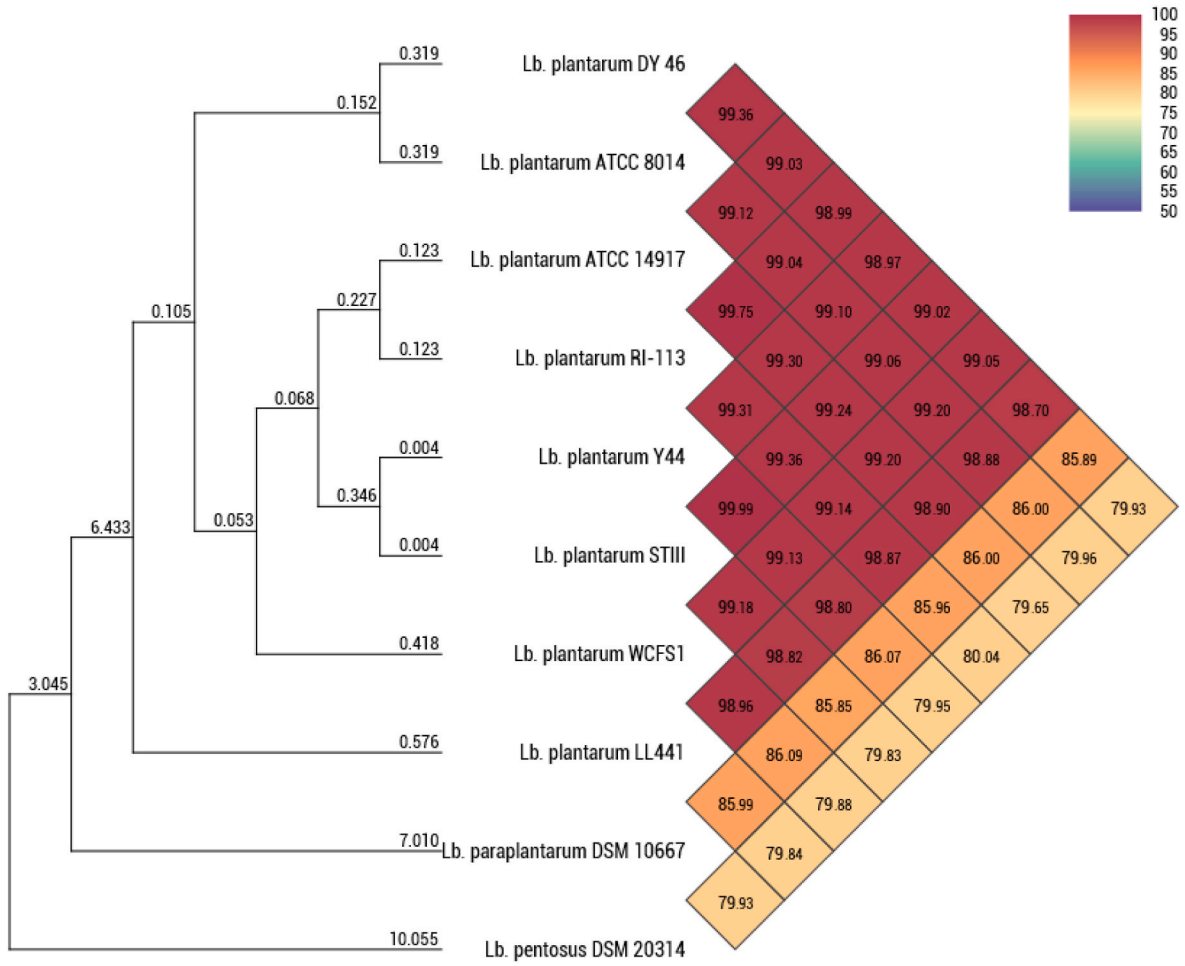

Fig. 3. Orthologous Average Nucleotide Identity (Ortho ANI) values of *Lactiplantibacillus plantarum* DY46 and other compared well-known *Lactiplantibacillus* species.

fructose-bisphosphate aldolase [EC:4.1.2.13], glucose-6-phosphate isomerase [EC:5.3.1.9], transketolase [EC:2.2.1.1] and phosphoketolase [EC:4.1.2.9/4.1.2.22] genes, which are encoding the key enzymes of EMP and PK pathways (Eiteman & Ramalingam, 2015). The genes encoding enzymes related in the intact EMP (Fig. S7.) and PK (Fig. S8.) pathways were predicted in the genome of the DY46 strain and listed in Table S21 (supplemental). Furthermore, 1-phosphofructokinase [EC:2.7.1.56] enzyme encoding *fruK* gene was detected which is used as the key gene for differentiation of hetero- and homofermentative lactobacilli species (Orita et al., 2008; J.; Zheng, Ruan, & Sun, 2015). On the other hand, Buron-Moles et al. (2019) reported that obligate heterofermentative strains lacked the 1-phosphofructokinase (PFK) enzyme, in addition to PFK absence, both obligately and facultative heterofermentative species were specifically characterized by the presence of L-arabinose isomerase, L-ribulose kinase and ribulose phosphate epimerase enzymes, respectively (Buron-Moles et al., 2019). Although these enzymes are not found in the genome of DY46, it could be predicted that DY46 has a facultative heterofermentative carbohydrate metabolism like other *Lb. plantarum* strains.

Lactiplantibacillus plantarum DY46 also possesses six L-Lactate dehydrogenase (*ldhL*) genes in the genome of DY46 which is smaller than what Liu et al. (2015); Gao et al. (2020) reported. The DY 46 appears to be capable of synthesizing only a single isomer of lactate, which may be due to a stable chromosomal deletion (Ferain, Garmyn, Bernard, Hols, & Delcour, 1994). Apart from the lactate dehydrogenase genes, the DY46 genome has several other pyruvate depletive enzymes which are responsible for the synthesis of other flavour compounds, such as acetaldehyde, acetoin, oxaloacetate, acetoin and ethanol (Gao et al., 2020; Papagianni, 2012). Moreover, the D-lactose and D-galactose fermenting capacity of the DY46 was confirmed by both *in vitro* and *in silico* analysis. The API results revealed that DY 46 was able to utilize glucose and lactose while, the BlastKOALA scanning results revealed that all enzymes of the Leloir metabolic pathway were present, together with 2 copies of the beta-galactosidase gene in the genome of the DY46.

The Leloir pathway enzyme encoding genes in the DY46 genome consist of one copy of galactokinase [EC. 2.7.1.6] gene, one copy of UDP-glucose-hexose-1-phosphate-uridylyltransferase [EC. 2.7.7.12] gene, four copies of UDP-glucose-4-epimerase [EC 5.1.3.2] and three copies of aldose-1-epimerase [EC 5.1.3.3] genes. The presence of genes encoding major enzymes of galactose metabolism and *in vitro* test results are hallmarks of the potential utilization of the DY46 as a dairy starter culture. Similar to *Lb. plantarum* strains of WCFS1(Kleerebezem et al., 2003), 5–2 (Liu et al., 2015), Y44 (Gao et al., 2020) and ZJ316 (P. Li et al., 2016), the *Lb. plantarum* DY46 genome did not encode all of the tricarboxylic acid cycle-related proteins, although certain genes were found and shown in Supplemental Table S21.

According to KEGG mapper reconstruction results, the genome of the DY46 strain encloses 203 genes that are related to central and another carbohydrate metabolism. The composition of those genes pools are as follows: 23 glycolysis/gluconeogenesis related genes, 8 TCA cycle-associated genes, 15 pentose phosphate pathway genes, 4 pentose and glucuronate interconversions related genes, 19 fructose and mannose metabolism genes, 19 galactose metabolism genes, 3 ascorbate and aldarate metabolism genes, 22 starch and sucrose metabolism genes, 27 amino sugar and nucleotide sugar metabolism genes, 28 pyruvate metabolism genes, 11 glyoxylate and dicarboxylate metabolism genes,

Table 3
Carbohydrate fermentation profile comparison between *Lactoplantibacillus plantarum* DY46 and previously studied isolates[1].

Sugar	Strain		
	DY46[2]	Y44[3]	ATTC14917[3]
Control	-	-	-
Glycerol	-	-	-
Erythritol	-	-	-
D-Arabinose	-	-	-
L-Arabinose	-	+	+
D-Ribose	+	+	+
D-Xylose	-	-	-
L-Xylose	-	-	-
Adonitol	-	-	-
Methyl-βD-xylopyranoside	-	-	-
D-Galactose	+	+	+
D-Glucose	+	+	+
D-Fructose	+	+	+
D-Mannose	+	+	+
D-Sorbose	-	-	-
D-Rhamnose	-	-	-
Dulcitol	-	-	-
Inositol	-	-	-
D-Mannitol	+	+	+
D-Sorbitol	-	+	+
Methyl-αD-mannopyranoside	+	+	+
Methyl-αD-glucopyranoside	-	-	-
N-Acetylglucosamine	+	+	+
Amygdalin	+	+	+
Arbutin	+	+	+
Esculin ferric citrate	+	+	+
Salicin	+	+	+
D-Cellobiose	+	+	+
D-Maltose	+	+	+
D-Lactose	+	+	+
D-Melibiose	+	+	+
D-Sucrose	+	+	+
D-Trehalose	+	+	+
Inulin	-	-	-
D-Melezitose	+	+	+
D-Raffinose	+	-	+
Amidon (Starch)	-	-	-
Glycogen	-	-	-
Xylitol	-	-	-
Gentiobiose	+	+	+
D-Turanose	-	+	+
D-Lyxose	-	-	-
D-Tagatose	-	-	-
D-Fucose	-	-	-
L-Fucose	-	-	-
D-Arabitol	-	-	-
L-Arabitol	-	-	-
Gluconate	+	+	+
2-Keto-gluconate	-	-	-
5-Keto-gluconate	-	-	-

1 The fermentation results are pointed out as follows; (+) positive, (−) negative.
[2] DY46 strain is used only in this study.
[3] Y44 and ATCC14917 strains were previously studied by Gao et al. (2020).

11 propanoate metabolism genes, 8 Butanoate metabolism genes, 3 C5-branched dibasic acid metabolism genes, 3 inositol phosphate metabolism genes. The gene number associated with carbohydrate metabolism is the same as *Lb. plantarum* Y44 (Gao et al., 2020). The number of carbohydrate-active enzyme genes of *Lb. plantarum* ZLP001 (Zhang et al., 2018) and *Lb. plantarum* KDLS1.0391 (Jia et al., 2017) were reported relatively low (119 and 190 genes, respectively) than the DY46 strain. However, there was no evidence of carbohydrate fermentation patterns that were found to be able to compare with the DY46 strain. In addition, as shown in Table 3, Y44 (24 sugars) and ATCC14917 (25 sugars) were able to metabolize a higher number of sugars than DY46. These differences may arise from the physiological and genetic adaptation of the strains to the ecological niches in which they were being isolated.

3.3. Bacteriocin biosynthesis

According to the results of the whole genome search of the DY46 strain against the BAGEL database, the gene cluster responsible for bacteriocin biosynthesis consists of 26 genes and its total length is approximately 24.2 kb (Fig. 4). In this gene cluster, transport-related genes, immunity protein and plantaricin precursor genes and several core genes (*Pln E, Pln F* and *pln K*) are encoded and all protein sequences are confirmed by protein BLAST (Table S1). *Lb. plantarum* DY46 strain was found to have the same core genes encoding Class II bacteriocins as ATCC8014 (Yu et al., 2020). The *plnEF* locus is widely distributed among *Lb. plantarum* strains isolated from various ecological niches. The well-studied *plnEF* loci have also been reported in *Lb. plantarum* WCFS1, NC8, JDM1, C11, V90, J51 and J23 strains, respectively (Tai et al., 2015). On the other hand, the isoelectric points (pI) and amino acid lengths of the *pln E* and *pln F* mature peptides that do not have the GG leader sequence that we detected (Table S2) are identical to *pln E* and *pln F* bacteriocins that were previously reported in WCFS1, NC8, J23, J51, C11 and V90 strains (Diep et al., 2009).

Normally, *pln J* and *pln K* peptides, which are subunits of plantaricin JK, are encoded in the same gene cluster/operon and more effective together, whereas in the present study only the *pln K* peptide was detected with 95.35% per cent identity (Todorov, 2009). Moreover, the pI (8.59) and length of the mature *pln K* peptide (28aa) detected have differed significantly from the mature plnK peptides (pI:10.52; 32aa) as previously reported in C11, NC8, V90 and WCFS1 (Diep et al., 2009). Apart from the core genes, the presence of secretion genes *pln H* (HlyD, accessory protein for ABC-transporter; (Accession no: WP_027821501.1), *pln G* (LanT, Bacteriocin ABC-transporter; WP_027821502.1) have been verified in the plantaricin gene cluster. Bacteriocin ABC transporters are involved in the transport of the mature peptide through the cell membrane, which is formed by deleting the leader peptide sequence from prebacteriocin (Havarstein, Diep, & Nes, 1995). The accessory protein (also called the accessory factor) is another necessary component for the ABC transporter system-dependent translocation process (Nes et al., 1996). Another gene cluster member identified is the putative Na^+/H^+ antiporter protein (orf00033: AFM80194.1), which sustainably maintains intracellular proton balance and leads to the enabling of ATP required for ABC transporters (Jia et al., 2017). In addition, the bacteriocin gene cluster contains genes encoding orf00020 (CAAX amino terminal protease family protein; EFK30757.1) and orf 00028 (bacteriocin immunity protein; WP_127526380.1) immunity proteins that play a role in protecting bacteria from their mature bacteriocins (Todorov, 2009). The other members of the bacteriocin biosynthetic gene cluster were listed in Supplemental Table S1.

3.4. Antibiotic resistance

Antibiotic susceptibility of the DY46 strain was evaluated as reported by the Clinical and Laboratory Standards Institute's performance standards. The zone of inhibition (ZOI) values of fourteen antibiotics tested against the DY46 strain was shown in Table S3 with resistome search match results. DY46 was found to be resistant (ZOI $\leq$14 mm) to methicillin (5 μg), oxacillin (1 μg), streptomycin (10 μg), vancomycin (30 μg), amikacin (30 μg), kanamycin (30 μg), azithromycin (15 μg), tetracycline (30 μg) and rifampacin (5 μg). Apart from these, DY46 was sensitive (ZOI $\geq$20 mm) to ampicillin (10 μg), carbenicillin (100 μg) and amoxicillin (25 μg), while it showed intermediate sensitivity (ZOI ~ 15–19 mm) to Penicillin G (10 U) and Erythromycin (10 μg).

The antibiotic resistance profile of the DY46 strain showed partial similarity with the previously reported *Lb. plantarum* profiles (Klarin, Larsson, Molin, & Jeppsson, 2019; C.; Sharma, Gulati, Thakur, & Pal, 2017; P.; Sharma, Tomar, Sangwan, Goswami, & Singh, 2016). There are no antibiotic resistance genes that have been found in both the ResFinder 4.1 and CARD databases. However, twenty-one antibiotic resistance genes which were shown in Table S3 have been detected with

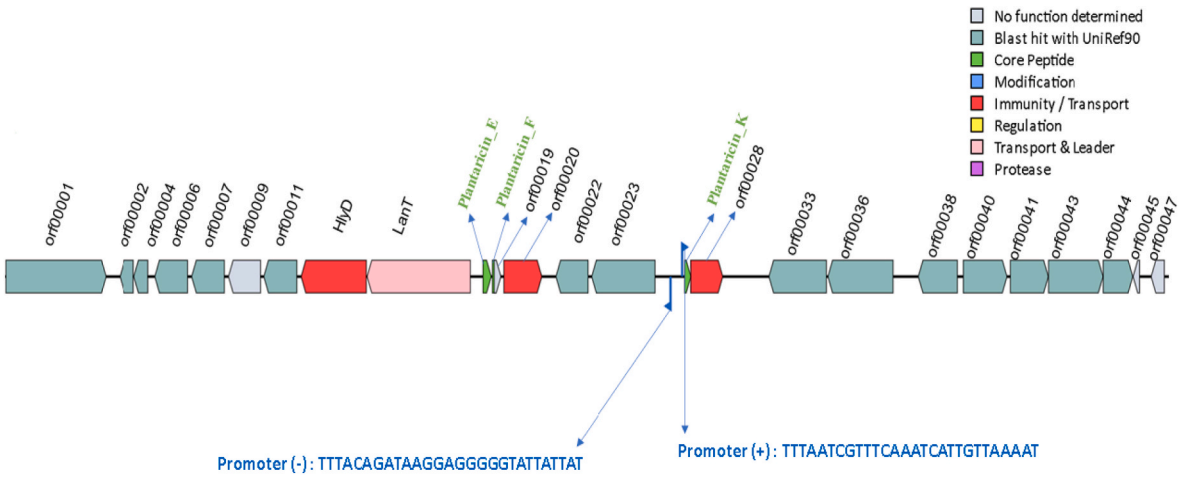

Fig. 4. The predicted gene cluster is responsible for the biosynthesis of Plantaricins by using the BAGEL4 webserver.

PATRIC 3.6.8 and KEGG databases. The identified genes were found to be related to β-Lactams (9), Streptomycin (2), Vancomycin (7), Macrolides (1), Tetracyclines (1) and Rifampicin (1). It is commonly accepted that *Lactobacillus* species have very high resistance to aminoglycosides. *Lb. plantarum* is known to be resistant to vancomycin due to its intrinsic peptidoglycan precursors consisting of D-lactate instead of D-alanine at the C-terminus (Álvarez-Cisneros & Ponce-Alquicira, 2018; Campedelli et al., 2019; Gueimonde, Sánchez, de los Reyes-Gavilán, & Margolles, 2013).

Besides, the *VanX* gene is highly specific for hydrolyzing D-ala-D-ala dipeptides and is a significant precursor of the cell wall (Liu et al., 2015). The vancomycin resistance genes were identified in the genome of the DY46 consists of phospho-N-acetylmuramoyl-pentapeptide-transferase [*mraY*; EC:2.7.8.13], alanine racemase [*alr*, EC:5.1.1.1], D-alanyl-D-alanine ligase [*ddl*, EC:6.3.2.4], UDP-N-acetylmuramoyl-tripeptide -D-alanyl-D-alanine ligase [*murF*, EC:6.3.2.10], UDP-N-acetylglucosamine–N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase [*murG*, EC:2.4.1.227], D-Ala-D-Ala carboxypeptidase [*vanY*, EC:3.4.17.14] and D-Ala-D-Ala dipeptidase [*vanX*, EC:3.4.13.22]. In addition, streptomycin resistance responsible *gibD* and *S12p* genes were detected. However, no specific genes were found for amikacin and kanamycin, even if resistance was present. This is because genotype and phenotype do not overlap completely (Zhang et al., 2012). Apart from these, *RlmA* (II) [23S rRNA (guanine(748)-N (1))-methyltransferase (EC 2.1.1.188)], *S10p* [SSU ribosomal protein S10p (S20e)], *rpoB* [DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)] genes detected which were related with macrolides, tetracyclines and rifampicin, respectively. Moreover, *mecA* [penicillin-binding protein 1A [EC:2.4.1.129, 3.4.16.4]], *pbp2a* [penicillin-binding protein 2A [EC:2.4.1.129, 3.4.16.4]] and *PenP* [beta-lactamase class A [EC:3.5.2.6]] major genes responsible for beta-lactam resistance were detected on the DY46 genome.

Methicillin and oxacillin resistance is known to be associated with penicillin-binding proteins. Interestingly, resistance to Penicillin G was observed in DY46, although *Lactobacillus* are known to be susceptible. Some authors have reported Penicillin G-resistance in recent years in some strains of *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, and *Lactobacillus plantarum*, which also confirms our study (Abriouel et al., 2015; Zheng et al., 2017). Because of the growing concern that foods and/or common bacteria may serve as potential reservoirs for antimicrobial resistance genes, probiotics must not carry transferable antimicrobial resistance genes to be used for humans or animals (Zhang et al., 2012). Because many *Lactobacillus* species have intrinsic resistance to many antimicrobial compounds, and such resistance is known not to be associated with any particular safety concerns. However, the intrinsic antibiotic resistance genes on the chromosome should not be flanked by integrases and/or transposases. As a result of Protein BLAST screening for antibiotic resistance genes detected in this study, no evidence of horizontal gene transfer was found (Supplemental Table S20).

3.5. Prophages and horizontal gene transfer

Prophage search results display 9 prophage regions (three intact, two questionable and four incomplete) found in the genome of the DY46 strain and summarized in Table 4. One of the three intact prophage regions showed similarity with Lactob_Sha1_NC_019489 (48.8 Kb), (region 1), and the other two like Lactob_phig1e_NC_004305 (39.9 Kb) and Staphy_SPbeta_like_NC_029119 (29.6 Kb), region 2 and region9, respectively. It was determined that Lactob_Sha_1 and Lactob_phig1e showed the highest protein matching among the identified prophages. These are the most common temperate prophages ever described in *Lb. plantarum* strains (Pei et al., 2020). All the prophage regions have attL/attR sequences and integrase except for region 6 (Paenib_PBL1c) and 7 (Bacill_vB_BtS_BMBtp14). In bacterial genomes, integrases are functional identifiers for phages, pathogenicity islands and integrative plasmids (Juhas et al., 2009; Liu et al., 2015).

Three integrases [PP_00611(region 1), PP_01193(region 2) and PP_03267 (region 9)] were determined in the identified intact regions. The location of attL/attR sequences varies within intact phages. The attL sequences in regions 2 and 9 are located upstream of the integrase, while the attR sequence of region 1 was found downstream of the integrase. Additionally, attL and attR sequences of phage 1 and phage 9 were identical, but the attL and attR sequences of phage 2 are different from them. The Intact phage region 1 extends from 558,178 bp to 607,042 bp of the genome and includes 58 protein-coding sequences containing all prophage components from PP_00554 (transposase) to PP_00611 (phage integrase). The intact region 2 extends from 1,217,186 bp to 1,257,125 bp of the genome and consists of 49 protein-coding sequences containing prophage components from PP_01193 (phage integrase) to PP_01241. Moreover, the intact region 3 (9) is located between 3,302,034 bp to 3,331,708 bp of the genome and consists of 33 protein-coding sequences containing prophage components from PP_03263 (protease) to PP_03295. Unlike previously reported for *Lb. plantarum* WCFS1(Ventura et al., 2003) and 5-2 strains (Liu et al., 2015), only the two intact phages (Sha1 and phig1e) were found to contain all packaging/head/tail gene clusters, DNA packaging genes and the lysis cassette. All components of the identified prophage elements have been listed in Tables S4–S12.

Horizontal gene transfer between bacteria can usually occur by natural competence or bacteriophage infection (Kleerebezem et al., 2003; Liu et al., 2015). The result of sequence homology screening revealed that most of the genes of the DY46 genome were homologous to *Lb. plantarum* genes and only 1.52% (49 genes) of total genes in the

Table 4
The predicted prophage regions of *Lactiplantibacillus plantarum* DY46 genome.

Region	Length	Completeness	Score	Region Position (bp)	Total Proteins	Most Common Phage (Number of matching proteins)	GC %	attL/attR sites	Integrase ORF start-stop
1	48.8 Kb	**Intact**	150	558178-607042	58	Lactob_Sha1_NC_019489 (27)	41.1	+	605879-607042
2	39.9 Kb	**Intact**	150	1217186–1257125	49	Lactob_phig1e_NC_004305 (20)	41.2	+	1217352–1218560
3	27.2 Kb	Questionable	70	2511806–2539007	21	Lactob_Sha1_NC_019489 (3)	42.4	+	2537850–2539007
4	24.6 Kb	Incomplete	30	2969719–2994363	9	Entero_IME_EF3_NC_023595 (2)	45.2	+	2981712–2982866
5	17.8 Kb	Incomplete	60	3002777–3020641	11	Bacill_Waukesha92_NC_025424 (2)	41.6	+	3018894–3019580
6	11.3 Kb	Questionable	80	3034765–3046067	21	Paenib_PBL1c_NC_048689 (2)	39.7	-	-
7	5.7 Kb	Incomplete	30	3136597–3142320	9	Bacill_vB_BtS_BMBtp14_NC_048640 (2)	38.7	-	-
8	11.7 Kb	Incomplete	50	3249993–3261724	15	Escher_ESCO5_NC_047776 (4)	38.9	+	3261137–3261724
9	29.6 Kb	**Intact**	150	3302034–3331708	33	Staphy_SPbeta_like_NC_029119 (4)	39.3	+	3313269–3314018

genome may have been gained thru horizontal gene transfer from other bacteria, e.g. *Pediococcus* spp., *Lactiplantibacillus pentosus*, *Lactiplantibacillus paraplantarum*, *Leuconostoc* sp., *Paucilactobacillus suebicus*, *Liquorilactobacillus hordei*, *Fructilactobacillus lindneri*, *Lacticaseibacillus paracasei*, *Loigolactobacillus coryniformis*, *Lactobacillus kefiranofaciens*, *Weissella jogaejeotgali*, *Lactobacillus sanfranciscensis*, *Lactiplantibacillus argentoratensis*, *Companilactobacillus* spp., *Levilactobacillus* spp., *Lentilactobacillus buchneri*, *Lactococcus lactis* subsp. *lactis* bv. *diacetylactis*, *Lactiplantibacillus daowaiensis*, *Latilactobacillus sakei*, *Bifidobacterium longum*, *Lapidilactobacillus mulanensis*, *Limosilactobacillus fermentum*, *Lactobacillus diolivorans*.

It was determined that most of the vertically transferred genes originated from *Lactobacillus* species are found in the microbiota of fermented vegetables. Among these 49 genes, 12 transposase genes (PP_02973, PP_02974, PP_02980, PP_02981, PP_03087, PP_03207, PP_03208, PP_03275, PP_03281, PP_03285, PP_03288, PP_03295) which were derived from recombination, repair and replication. Moreover, all the genes considered horizontally transferred were phage related and summarized in Tables S13–19.

3.6. Probiotic properties

When new probiotic strains are discovered, certain characterization tests are required to confirm probiotic properties. Therefore, probiotic characterization tests were performed to confirm the probiotic properties of the DY46. The β-haemolysis test results showed that DY46 does not have β-hemolytic activity. The cell surface hydrophobicity of DY46 is characterized by using xylene. As shown in Fig. 5A, the cell surface hydrophobicity of DY46 appears to increase in direct proportion to the bile salt concentration. Cell surface hydrophobicity of the DY46 was determined as 33%, 38.5% and 46.1% at 3, 5 and 10 g/L bile salt concentrations, respectively. The cell surface hydrophobicity of the control sample was found to be at 4.38%. However, similar to present work, it has been reported in previous studies that some lactobacilli, including *Lb. acidophilus* and *Lb. johnsonii* strains displayed surface hydrophobicity as low as 2–5% (Rijnaarts, Norde, Bouwer, Lyklema, & Zehnder, 1993; Schillinger, Guigas, & Holzapfel, 2005). Kaushik et al. (2009) reported that such large differences in cell surface hydrophobicity could occur due to differences in the expression level of cell surface proteins, depending on environmental conditions and/or bacterial strain.

Auto-aggregation is an important bacterial characteristic in different ecological niches, especially in human and animal mucosa where probiotics confer health benefits. The auto-aggregation capacity is an important factor for maintaining sufficient numbers of probiotic strains under adverse conditions of the oral cavity and gastrointestinal tract. The cellular auto-aggregation test results presented in Fig. 5B revealed that there is an inverse correlation between auto-aggregation and bile salt concentrations. The auto-aggregation ability of DY46 was determined as 85.7% for the control sample, while it was determined as 84.69%, 79.05% and 51.35% for bile salt concentrations of 3, 5 and 10 g/L, respectively. Li, Liu, Dong, Zhou, and Wang (2015) reported auto-aggregation ranged from 0.86 to 65.15% in different *Lb. fermentum* strains isolated from various Chinese fermented foods (Li et al., 2015). Ramos, Thorsen, Schwan, and Jespersen (2013) reported an auto-aggregation value between 18.08 and 20.94% in cocoa originated *Lb. plantarum* strains. Moreover, Goel et al. (2020) reported 52.91% and 72.84% auto-aggregation ability for two different *Lb. plantarum* strain isolated from Indian fermented foods (Goel et al., 2020). Similar to the present study, Aslim, Onal, and Beyatli (2007) observed a reduction of auto-aggregation capacity in the presence of bile in *Lb. delbrueckii* subsp. *bulgaricus* strains. The fact that the DY46 showed an auto-aggregation capacity of 51.35% at a bile salt concentration close to the real gastrointestinal tract (GIT) environment is significant to prove its probiotic property. Based on the antimicrobial activity test results, the DY46 displayed an apparent zone of inhibition (> 5 mm) against *K. pneumoniae* (ATCC 13883), *P. vulgaris* (ATCC 8427) and *S.enterica* sv. *Typhimurium* (ATCC 14028) whereas, there is no inhibition zone observed against other test strains. The lack of *Pln J* peptide in the genome of the DY46 strain brings confounding factor of whether inhibition zones achieved against test pathogens might also be due to the organic acids produced by DY46.

It is necessary to test probiotic candidates against acid and bile salts to determine their resistance under inevitable conditions of the human gastrointestinal tract (GIT) (Angmo et al., 2016). The growth kinetics of DY46 at different pH (2, 3, 4, 5 and 7) conditions at 30 and 37 °C are shown in Figs. S3 and S4. No or ignorable level of growths seen at pH=2 and pH=3 conditions for both 30 and 37 °C. Similar findings were also reported that cell density of Lactobacilli are significantly influenced by low pH, especially at 1.5 and 2 (Angmo et al., 2016; Guo, Kim, Nam, Park, & Kim, 2010).

The duration of lag phase achieved was pH=7<pH=5<pH=4 in both 30 and 37 °C. The maximum specific growth rates achieved at both temperatures were as follows pH=7>pH=5>pH=4. Similarly, final cell turbidities achieved were pH=7>pH=5>pH=4. The μ_{max} levels achieved at 30 and 37 °C were slightly different with the latter providing better growth performance. This perhaps relates to the probiotic potential of DY46 that can proliferate at a body temperature of 37 °C. The decreasing μ_{max} values and poor or no growth seen at lower pH values are perhaps due to the cellular machinery effort to maintain relatively neutral pH values in the cytoplasm at the expenditure of ATP to push thru H^+ cations across the outside of the membrane (Axelsson, 2004).

The growth kinetics of DY46 at different bile (0.3%, 0.5%, 1% and

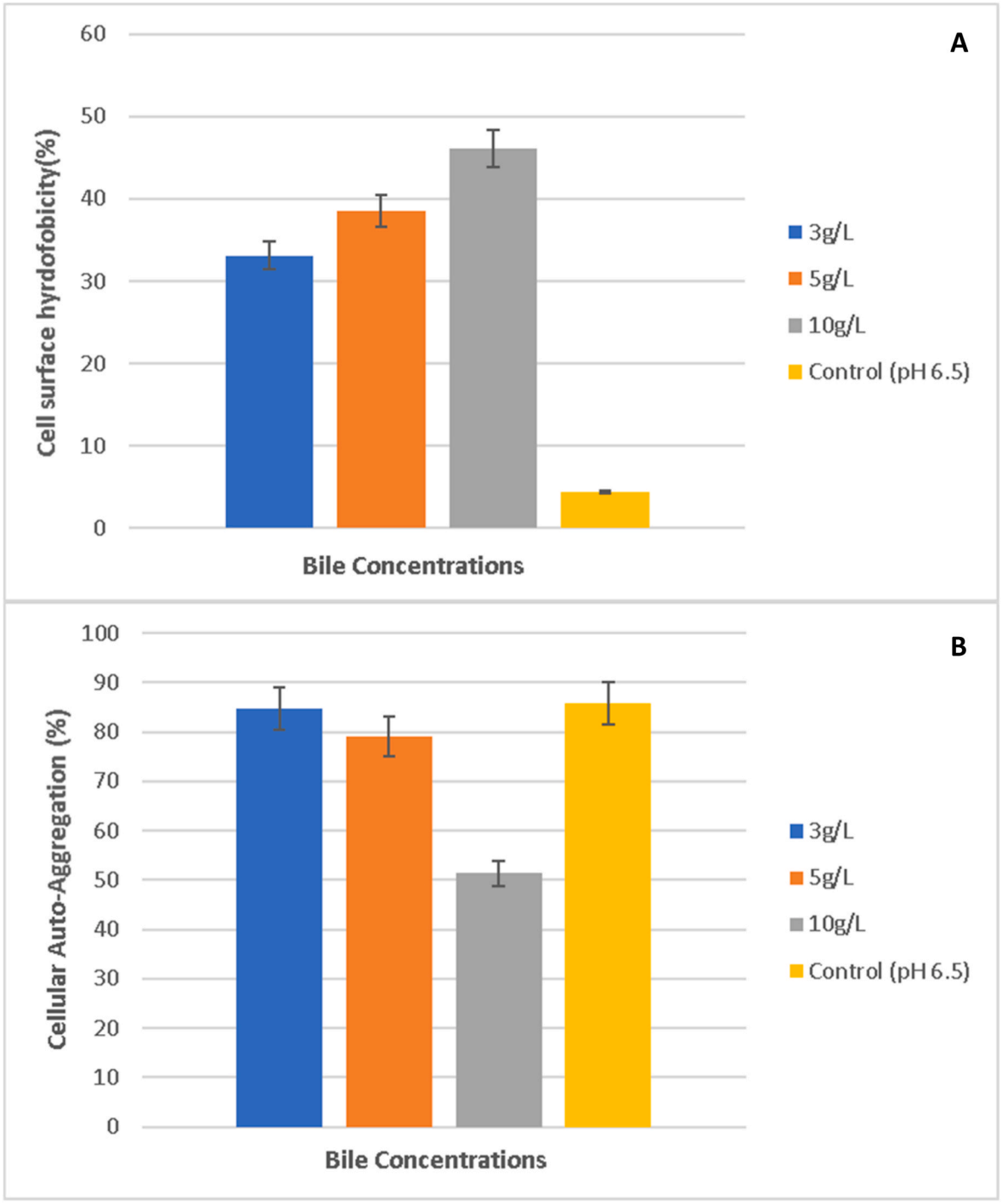

Fig. 5. Graphical presentation of cell surface hydrophobicity (A) and cellular auto-aggregation (B) test results.

control) conditions at 30 and 37 °C are shown in Figs. S5 and S6. A significantly lower lag phase duration was observed at 37 °C vs 30 °C. The μ_{max} values achieved at 37 °C were as follows Control (No bile)> 0.3% bile>0.5% bile>1% bile. Final cell densities achieved at 37 °C were Control (No bile)>0.3% bile>0.5% bile=1% bile. We speculate that the bile salt exerts certain stress on the cell and is triggered by temperature. Nevertheless, DY46 can still proliferate to remarkable cell concentrations as measured with microplate reader across all bile levels even at 37 °C. It was interesting to note that although longer lag phases were achieved for all bile treatments at 30 °C, the cells further caught up with higher μ_{max} values achieved compared to 37 °C. This shows DY46 perhaps could better tolerate bile salt stress at a lower temperature which can be supported by the observation of similar final cell densities across all bile concentrations at 30 °C.

Overall, better growth kinetics achieved at 37 °C for pH conditions tested though lower pH values reduced or decreased growth completely. Although a shorter lag phase was seen at 37 °C with all bile concentrations evaluated, DY46 cells better-tolerated bile salt at 30 °C with higher μ_{max} and final cell concentrations obtained. The DY46 can be resisting and proliferating under adverse conditions with moderately lower pH values and bile concentration mimicking the human GIT.

4. Conclusions

In summary, genomic, physiological and probiotic characterization of *L. plantarum* DY46 isolated from shalgam has been performed for the determination of specific properties of this novel strain. The proposed strain's genomic features, carbohydrate fermentation patterns, bacteriocin biosynthesis, antibiotic resistance situation, prophages and related horizontal gene transfer and probiotic properties have been briefly discussed in separate threads and the following conclusions can be proclaimed:

(1) Genome analysis revealed this strain follows a facultative heterofermentative sugar metabolism where hexoses are cleaved thru glycolysis versus pentoses are hydrolyzed via the pentose phosphate pathway.
(2) Genome evidence predicted that DY46 could biosynthesize *Pln E*, *Pln F*, *Pln K* peptides but, the lack of *Pln J* peptide in the genome of DY46 brings the conflictive factor of whether inhibition zones achieved against test pathogens might also be due to the organic acids produced by DY46. Therefore, further investigations are necessary for bacteriocins by using proteomic approaches.
(3) In total, 9 prophage regions (3 intact, 2 questionable and 4 incomplete) have been found in the genome of the DY46. Related to this, 49 genes have been determined as horizontally transferred from different bacteria which 12 of them are transposase genes. On the other hand, it is understood that all detected resistome in the DY46 genome are intrinsic originated.
(4) DY46 is found to be tolerant to acid and bile concentrations mimicking human gastrointestinal conditions.
(5) Overall, *L. plantarum* DY46 is a promising bacterium possessing certain probiotic traits confirmed by *in vitro* analysis and perhaps a potential dietary supplement candidate that might provide therapeutic benefits to the host. It is understood that further *in vivo* studies will be proper for absolute confirmation of its therapeutic and functional features.

Author statement

Ahmet E. Yetiman: Conceptualization, Methodology, Data Curation, Writing-Review&Editing, Investigation, Visualization, Project Administration And Funding Acquistion, **Abdullah Keskin:** Investigation, **Büşra Nur Darendeli:** Investigation, **Seyfullah Enes Kotil:** Investigation, **Fatih Ortakci:** Conceptualization and Writing-Review&Editing, **Mahmut Doğan:** Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2021.101499.

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