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Complete genome sequence of *Ligilactobacillus agilis* LDTM47, bacteriocin-producing lactic acid bacteria isolated from broiler gastrointestinal tract

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Abstract

Ligilactobacillus agilis LDTM47 was isolated from gastrointestinal tract of broiler chickens from a farm associated with Chung-Ang University (Anseong, Korea). *Ligilactobacillus* are Gram-positive lactobacilli associated with the intestinal tracts of vertebrates. Lactic acid bacteria are considered to have a generally rec¬ognized as safe (GRAS) status from the Food and Drug Administration (FDA). The genome of *Lig. agilis* LDTM47 was 2,144,466 bp long assembled into 1 contig, with 2,131 protein-coding sequences, 90 tRNA genes, 24 rRNA genes, and a guanine + cytosine (GC) content of 41.9%. Strain LDTM47 was selected based on its inhibitory activity against *Listeria monocytogenes* during isolation. The genome analysis of LDTM47 revealed genes encod¬ing the bacteriocin core peptides and associated export proteins. This study analysis confirmed bacteriocin stability (instability index 1.32) and susceptibility to proteolytic enzymes, indicating non-toxicity. These findings suggest their potential as a safe alternative to antibiotics for controlling pathogens.

Keywords: *Ligilactobacillus agilis*, Postbiotics, Bacteriocin, Antibiotic alternatives, Genome announcement

Postbiotics are bioactive cellular components that are not classified as probiotics, prebiotics, or paraprobiotics, and may contain purified or a mixture of soluble factors, metabolic products and/or by-products, and other cell components that confer a beneficial health effect on the host. Bacteriocins, defined as antimicrobial peptides synthesized by the ribosome, are considered postbiotics that may have beneficial effects on the host, directly or indirectly [1]. The proteinaceous nature of these substances makes them susceptible to hydrolysis by endogenous proteolytic enzymes from animals or humans and exerts antibacterial, antibiofilm, or potentially anti-cancer properties [2]. Thus, bacteriocins are becoming increasingly important in the dairy and feed sectors for biopreservation and as substitutes for antibiotics. In contrast, ISAPP defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [3]. Although probiotics are generally regarded as safe (GRAS), there is still an imminent risk of transmission of harmful genes such as antimicrobial

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Kim GB.
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Formal analysis: Eum B, Elnar AG, Jang Y, Kim GB.
Methodology: Eum B, Elnar AG, Kim GB.
Software: Eum B, Elnar AG, Jang Y, Kim GB.
Validation: Kim GB.
Investigation: Eum B.
Writing - original draft: Eum B, Elnar AG, Jang Y.
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Ethics approval and consent to participate
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resistance and virulence factor genes. Meanwhile, postbiotics offer several benefits, such as safer delivery, extended shelf life, and less risk of acquiring and spreading resistance genes and other harmful factors [4].

Different classes of bacteriocins include Class I and Class II bacteriocins, consisting of small molecular-size (≤ 10 kD), heat-stable bacteriocins, and Class III bacteriocins, comprised of small, heat-labile bacteriocins. Class I is further divided into subclass Ia and Ib corresponding to 'lantibiotics' and 'circular bacteriocins,' while Class II is divided into subclass IIa to IId, corresponding to 'pediocin-like bacteriocins', 'two-peptide bacteriocins', 'leaderless bacteriocins' and 'non-pediocin-like single peptide bacteriocins', respectively. Lastly, Class III can either be 'bacteriolysin bacteriocin' or 'non-lytic bacteriocin' [4]. The extensive range of bacteriocins provides prospects for investigating alternatives to traditional antimicrobials and requires thorough research to accurately define and apply these bioactive peptides with great precision.

The bacteriocin-producing *Ligilactobacillus agilis* LDTM47 strain was isolated from the gastrointestinal tract contents (jejunum and ileum) of 5-week-old broilers from a farm affiliated with Chung-Ang University (Anseong, Korea). *Lig. agilis* LDTM47 is a Gram-positive, facultatively anaerobic, and rod-shaped bacteria. Most lactic acid bacteria are non-motile; however, *Lig. agilis* exerted motility and was later observed to be flagellated [5]. Generally, *Lig. agilis* LDTM47 was cultured aerobically in de Man, Rogosa, and Sharpe (MRS) medium (BD Bacto) at 37℃ for 24 h [6]. The genomic DNA was sequenced using the Pacific Biosciences (PacBio, Menlo Park, CA, USA) RSII Single Molecule Real-Time (SMRT) platform and a 20-kb SMRKbell™ template library. The PacBio reads were assembled using the FALCON 0.5 program *de novo*. Functional categorization and annotation via Rapid Annotation using Subsystem Technology (RAST) (http://rast.nmpdr.org/) and CLgenomics™ ver. 1.55 software and Cluster of Orthologous Groups (COG) derived from the EZBioCloud data were performed [4]. Functional annotation of protein-coding genes was performed using PRODIGAL ver. 2.6.2 software (Fig. 1) [7]. Putative bacteriocin genes were verified *in silico* using the BAGEL4 software (http://bagel4.molgenrug.nl/). The *Lig. agilis* LDTM47 whole genome sequencing (Fig. 2) showed a 2,144,466 base pair genome with a guanine + cytosine (GC) content of 41.9%. The genome was composed of a single contig with an N50 value of 2,144,466 bp. The genome comprises 2,131 protein-coding genes, 90 tRNA genes, and 24 rRNA genes, as shown in Table 1.

BAGEL4 analysis revealed that *Lig. agilis* LDTM47 harbors the core peptide gene, immunity, and transport genes for bacteriocin production (Fig. 3). One open reading frame (ORF) was predicted, encoding the bacteriocin core peptide with the amino acid sequence of MENKKK LTKADLAKVTGGSRY**YGNGV**TCGKHKCTVNWGQAWTCGVNRLANFGHGNC. The 'YGNGV' motif is associated with pediocin-like bacteriocin [8], suggesting that LDTM47 bacteriocin is a Class IIa bacteriocin. The *lanT* encodes the AbpT bacteriocin export accessory protein [9], and the *abc* encodes the import adenosine triphosphate (ATP)-binding protein FhuC [10]. Additionally, *entA* encodes the bacteriocin immunity protein. *In silico* characterization revealed that LDTM47 bacteriocin is stable with an instability index (II) of 1.32 (https://web.expasy.org/cgi-bin/protparam/protparam). Additionally, the bacteriocin was predicted to be susceptible to a number of proteolytic enzymes, including Arg-C proteinase, Asp-N endopeptidase, enterokinase, pepsin, proteinase K, and trypsin (https://web.expasy.org/cgi-bin/peptide_cutter/peptidecutter.pl). A BLASTp search of the LDTM47 amino acid sequence against *L. agilis* (taxid:1601) yielded only a limited number of significant alignments, indicating that the bacteriocin has received relatively little research interest thus far. Further, the sequence was searched in the RCSB Protein Data Bank and revealed the most relevant sequence identity (63%) with leucocin A, having 13 amino acid differences in (K20R, H27T, T29G, S31H, G32K, S34T, E39Q, F41W, S42T, A43C, H46C,

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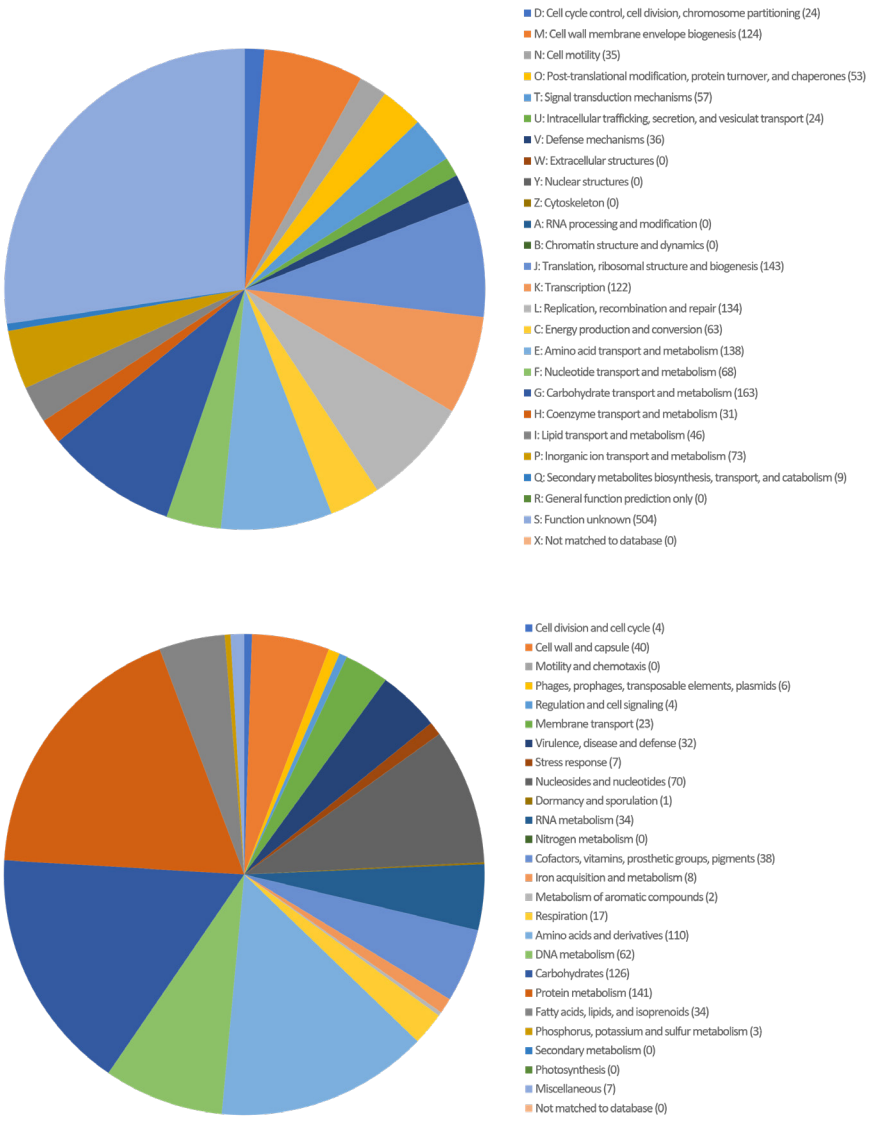

Fig. 1. Distribution by KEGG annotation (A) and Cluster of Orthologous Group (B) based on the functional classification of whole genome of *Ligilactobacillus agilis* LDTM47. KEGG, Kyoto Encyclopedia of Genes and Genomes.

G51N, and N53H). To our knowledge, only four *Lig. agilis* strains of chicken origin have been studied. Out of these strains, only one was found to produce a bacteriocin (garvicin), implying the need for further investigation on these bacteriocins.

Preliminary characterization of the physicochemical properties of LDTM47 bacteriocins revealed temperature and pH stability (data not shown) consistent with their Class IIa classification and *in silico* characterization of their stability, suggesting their safety and suitability in food and feed system applications. Although *Lig. agilis* LDTM47 strain lacks resistance to low pH and bile acids, rendering it challenging for probiotic development, its bacteriocin production may have potential applications as postbiotics, as biopreservation, and antibiotic alternatives.

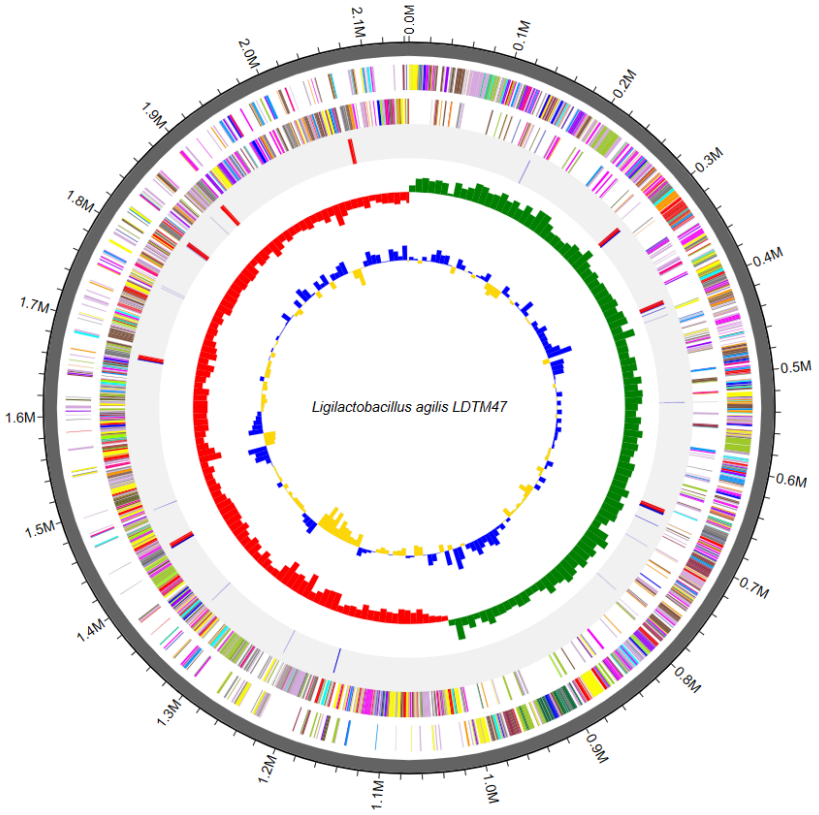

Fig. 2. Circular genome map of *Ligilactobacillus agilis* LDTM47. Circles represent the following characteristics from the outermost circle to the center: (1) contig information, (2) coding sequences on forward strand, (3) coding sequences on reverse strand, (4) transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), (5) GC skew, and (6) GC ratio. G, guanine; C, cytosine.

Table 1. Genome characteristics of *Ligilactobacillus agilis* LDTM47

Attribute	Value
Genome size (bp)	2,144,466
GC content (%)	41.9
No. of contigs	1
Total genes	2,245
Protein-coding gene	2,131
tRNA	90
rRNA	24
Plasmids	0
GenBank Accession No.	CP141636

G, guanine; C, cytosine.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The sequence obtained in this Whole Genome Shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number CP141636. The BioProject accession number is SAMN38724984 and the Biosample accession number is PRJNA1050031.

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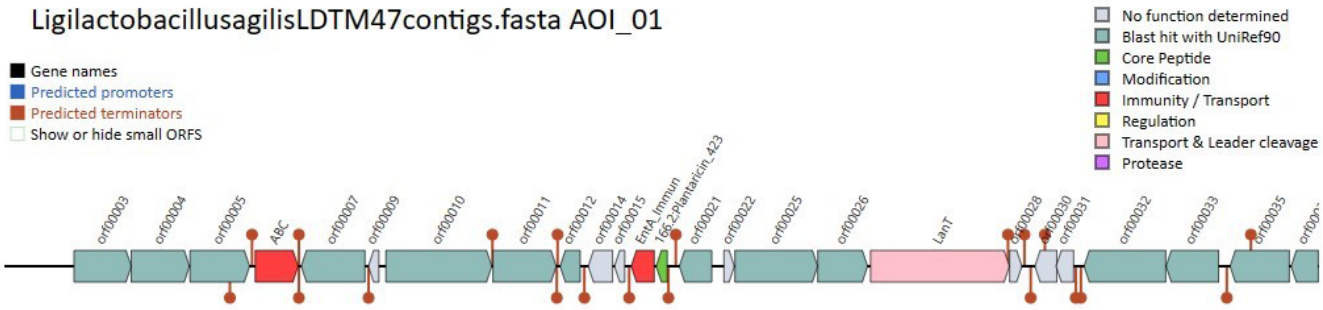

Fig. 3. Predicted bacteriocin gene cluster in Ligilactobacillus agilis LDTM47 genome showing a single open reading frame (ORF) for plantaricin_423 core peptide (green) using BAGEL4 software.

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