JAST (Journal of Animal Science and Technology) TITLE PAGE
Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Genome Announcement
Article Title (within 20 words without abbreviations)	Complete genome sequence of bacteriocin-producing *Ligilactobacillus salivarius* B4311 isolated from fecal samples of broiler chicken with anti-listeria activity
Running Title (within 10 words)	Genome of *Ligilactobacillus salivarius* B4311, bacteriocin-producing strain
Author	Subin Han[1†], Arxel G. Elnar[1†], Chiwoong Lim[1], Geun-Bae Kim[1]
Affiliation	[1] Department of Animal Science and Technology, Chung-Ang University, Anseong 17546, Korea
ORCID (for more information, please visit https://orcid.org)	Subin Han (https://orcid.org/0000-0001-6658-2822) Arxel G. Elnar (https://orcid.org/0000-0002-2716-4924) Chiwoong Lim (https://orcid.org/0000-0002-6272-4464) Geun-Bae Kim (https://orcid.org/0000-0001-8531-1104)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This research was supported by the Chung-Ang University Graduate Research Scholarship (Academic Scholarship for College of Biotechnology and Natural Resources) in 2021.
Acknowledgements	Not applicable.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Kim GB Data curation: Han S, Elnar AG Formal analysis: Elnar AG, Kim GB Methodology: Elnar AG, Kim GB Software: Elnar AG, Chiwoong Lim, Kim GB Validation: Kim GB Investigation: Elnar AG Writing - original draft: Han S, Elnar AG Writing - review & editing: Han S, Elnar AG, Chiwoong Lim, Kim GB [†]Han S and Elnar AG contributed equally to this work.
Ethics approval and consent to participate	This article does not require IRB/IACUC approval because there are no human and animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Geun-Bae Kim
Email address – this is where your proofs will be sent	kimgeun@cau.ac.kr
Secondary Email address	
Address	Department of Animal Science and Technology, Chung-Ang University, Anseong 17546, Korea
Cell phone number	
Office phone number	+82-31-670-3027
Fax number	+82-31-676-5986

Abstract

Ligilactobacillus is a genus of Gram-positive lactobacilli commonly found in the intestinal tracts of vertebrates. It has been granted a Qualified Presumption of Safety (QPS) status from the European Food Safety Authority (EFSA). One specific strain, *Ligilactobacillus salivarius* B4311, was isolated from fecal samples of broiler chickens from a farm associated with Chung-Ang University (Anseong, Korea). This strain was observed to have inhibitory effects against *Listeria monocytogenes*. In this paper, we present the complete genome sequence of *Lig. salivarius* B4311. The whole genome of strain B4311 comprises 2,071,255 bp assembled into 3 contigs representing a chromosome, *repA*-type megaplasmid, and small plasmid. The genome contains 1,963 protein-coding sequences, 22 rRNA genes, and 78 tRNA genes, with a guanine + cytosine (GC) content of 33.1%. The megaplasmid of strain B4311 was found to contain the bacteriocin gene cluster for salivaricin P, a two-peptide bacteriocin belonging to class IIb.

Keywords: *Ligilactobacillus salivarius*, probiotics, bacteriocin, *Listeria monocytogenes*

Main Text

Bacteriocin production in lactic acid bacteria (LAB) has been consistently gaining attention owing to its potential as a viable alternative to antibiotics. Bacteriocins are ribosomally-synthesized peptides secreted by the producing strain. These peptides can have either a narrow or broad spectrum of activity, which indirectly determines the niche of the producing strain. The production of bacteriocins is generally viewed as a positive trait as it enables the producing strain to hinder potential competitors in the immediate environment as well as inhibit potentially harmful microorganisms (1). The proteinaceous nature of bacteriocins renders them suitable for human use as they can be inactivated by digestive proteases. With the rapid development of antimicrobial drug resistance in microorganisms (2), research efforts focused on developing alternative solutions must be prioritized.

Commonly associated with vertebrate hosts, *Ligilactobacillus* is a genus of lactic acid bacteria composed of members that are homofermentative, non-motile, and urease-positive. Their ability to

survive in gastric acid conditions and their Qualified Presumption of Safety (QPS) status from the European Food Safety Authority (EFSA) (3) make them popular choices for probiotics. Furthermore, the production of various antimicrobial salivaricins among strains of *Lig. salivarius* is well accounted for the development of probiotic strains (4). In the present study, we report the genome analysis of a bacteriocin-producing *Ligilactobacillus salivarius* (formerly *Lactobacillus salivarius*) strain B4311, which was isolated from fecal samples collected from broiler chickens.

Strain B4311 was routinely cultured in de Mann, Rogosa, Sharpe (MRS, BD Difco, USA) broth supplemented with 0.05% L-cysteine, and incubated aerobically at 37°C for 24 h. Genomic DNA was extracted using the MagAttract HWM DNA Kit (Qiagen, Germany) and quantified using Qubit ds DNA HS assay kit (Invitrogen, USA) with the Epoch™ Spectrometer (BioTek). The genome was sequenced using the Pacific Biosciences (PacBio, USA) Sequel II platform. *De novo* assembly of the sequence reads was performed using the PacBio SMAR Analysis program (ver. 2.3.0). Functional annotation of the genome was performed using PRODIGAL ver. 2.6.2 (5) software and compared against protein databases (SwissProt, KEGG, SEED, EggNOG). Rapid annotation was employed using Subsystem Technology (RAST) with default parameters (https://rast.nmpdr.org/). Transfer RNAs (tRNA) and non-coding ribosomal RNAs (rRNA) were identified using tRNAscan-SE ver. 1.3.1 (6) and INFERNAL ver. 1.1.3 (7), respectively.

The complete genome of *Lig. salivarius* B4311 is 2,071,255 bp which is assembled into three contigs: a single chromosome (1,801,655 bp), one megaplasmid (247,930 bp), and a small plasmid (21,670 bp) with a guanine + cytosine (GC) content of 33.1%. In addition, the genome contains 1,963 protein-coding sequences, 22 rRNA genes, and 78 tRNA genes. The genome features and circular maps of strain B4311 are presented in Table 1 and Fig. 1, respectively. Antimicrobial resistance genes, specifically for tetracycline and glycopeptides, were also detected via Resistance Gene Identifier (RGI, https://card.mcmaster.ca/home). Among the 1,963 CDS, 1,241 were predicted with biological functions associated with cell cycle (n = 23), cell wall and motility (n = 116), cellular response (n = 69), DNA processing (n = 154), RNA processing (n = 119), protein processing (n = 202), defense mechanism (n

= 31), energy production (n = 63), and transport and metabolism (n = 464). Additionally, 61 putative genes were detected with putative functions including stress response, DNA and RNA processing, antibiotic resistance, periplasm signaling, acetylation, amino acid transport, and production of enzymes including various hydrolases, methyltransferases, and transport proteins.

In silico analysis of the B4311 genome using BAGEL4 online program (http://bagel4.molgenrug.nl/) revealed the presence of a bacteriocin gene cluster for salivaricin P, a family of two-peptide bacteriocins belonging to class IIb. This bacteriocin family was originally discovered from *Lig. salivarius* DPC6005 (6) and is commonly produced among strains of *Lig. salivarius* isolated from animals intestines (8). The salivaricin P gene cluster is located in the *repA*-type megaplasmid. Although the presence of megaplasmids is considered a typical feature of *Lig. salivarius*, variations exist among megaplasmid-encoded traits, including contingency metabolism genes (i.e., assimilation of sugars) and the presence or absence of bacteriocin genes, which provides a competitive advantage.

The genetic architecture of the bacteriocin gene cluster (Fig. 2) revealed the presence of two open reading frames (ORFs) encoding the salivaricin P chain A and chain B. The two peptide chains share a homologous sequence. Located downstream of the genes for the bacteriocin peptides are two ORFs encoding a histidine kinase and *AbpR*, which function as regulator proteins (9). These are followed by *AbpIM* which encodes an immunity protein. These five ORFs are flanked by two *comC* genes, which have been reported as competence-stimulating peptide precursors in streptococci (10). At the 3' end of the gene cluster, two export proteins, *LanT* and *HlyD* were detected, encoding *AbpT* and *AbpD* bacteriocin export accessory proteins, respectively. Several ORFs encoding bacteriocin core peptides (i.e., lactacin F and plantaricins) were also detected. However, the similarity of these genes with the reference was poor, suggesting that the translated peptides might be inactive.

Production of active bacteriocins was demonstrated by spot-on-lawn assay against *Listeria monocytogenes* ATCC 19114 and ATCC 19115 strains. The cell-free supernatant of strain B4311 successfully inhibited the growth of *Lis. monocytogenes* (unpublished data), a common foodborne

pathogen associated with raw and unpasteurized milk and the causative agent of listeriosis. The genomic information presented in this study confirms the ability of strain B4311 to elaborate bioactive peptides, which can have valuable applications in the food and animal industries.

Nucleotide sequence accession number

The sequence obtained in this Whole Genome Shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number CP117983-CP117985. The BioProject accession number PRJNA932943 is and the Biosample accession number is SAMN33215311.

Acknowledgments

This research was supported by the Chung-Ang University Graduate Research Scholarship (Academic Scholarship for College of Biotechnology and Natural Resources) in 2021.

Availability of data and material

Upon a reasonable request, the datasets of this study can be requested from the corresponding author.

105 **References**

106 1. Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: a probiotic trait? Appl
107 Environ Microbiol. 2012;78(1):1-6.

108 2. Darbandi A, Asadi A, Mahdizade Ari M, Ohadi E, Talebi M, Halaj Zadeh M, et al.
109 Bacteriocins: Properties and potential use as antimicrobials. J Clin Lab Anal.
110 2022;36(1):e24093.

111 3. Hazards EPoB, Koutsoumanis K, Allende A, Alvarez-Ordonez A, Bolton D, Bover-Cid S,
112 et al. Update of the list of QPS-recommended biological agents intentionally added to food
113 or feed as notified to EFSA 12: suitability of taxonomic units notified to EFSA until March
114 2020. EFSA J. 2020;18(7):e06174.

115 4. Dec M, Stepien-Pysniak D, Puchalski A, Hauschild T, Pietras-Ozga D, Ignaciuk S, et al.
116 Biodiversity of *Ligilactobacillus salivarius* Strains from Poultry and Domestic Pigeons.
117 Animals (Basel). 2021;11(4).

118 5. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic
119 gene recognition and translation initiation site identification. BMC Bioinformatics.
120 2010;11:119.

121 6. Barrett E, Hayes M, O'Connor P, Gardiner G, Fitzgerald GF, Stanton C, et al. Salivaricin P,
122 one of a family of two-component antilisterial bacteriocins produced by intestinal isolates of
123 *Lactobacillus salivarius*. Appl Environ Microbiol. 2007;73(11):3719-23.

124 7. Nawrocki EP, Eddy SR. Infernal 1.1: 100-fold faster RNA homology searches.
125 Bioinformatics. 2013;29(22):2933-5.

126 8. Messaoudi S, Manai M, Kergourlay G, Prevost H, Connil N, Chobert JM, et al. Lactobacillus
127 salivarius: bacteriocin and probiotic activity. Food Microbiol. 2013;36(2):296-304.

128 9. Barbour A, Wescombe P, Smith L. Evolution of Lantibiotic Salivaricins: New Weapons to
129 Fight Infectious Diseases. Trends Microbiol. 2020;28(7):578-93.

130 10. L.S. H, R. H, P. G. Natural Competence in the Genus *Streptococcus*: Evidence that
131 Streptococci Can Change Pherotype by Interspecies Recombinational Exchanges. Journal of
132 Bacteriology. 1997;179(21):6589-94.

133

Legends of Tables and Figures

Table 1. Genome features of *Ligilactobacillus salivarius* B4311.

Fig. 1. Circular map of *Ligilactobacillus salivarius* B4311 genome. G, guanine; C, cytosine; CDS, coding sequences. 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.

Fig. 2. Predicted bacteriocin gene cluster in *Ligilactobacillus salivarius* B4311genome showing two ORFs for salivaricin P core peptides. ORF, open reading frame.

134

135 **Table 1**. Genome features of *Ligilactobacillus salivarius* B4311.

Attribute	Value			
	Chromosome	Megaplasmid	Plasmid	Total
Size (bp)	1,801,655	247,930	21,670	2,071,255
GC content (%)	33.24	32.25	33.68	33.1
No. of contigs	1	1	1	3
Total genes	1,768	273	22	2,063
Protein-coding gene	1,668	273	22	1,963
tRNA	78	-	-	78
rRNA	22	-	-	22

136
137

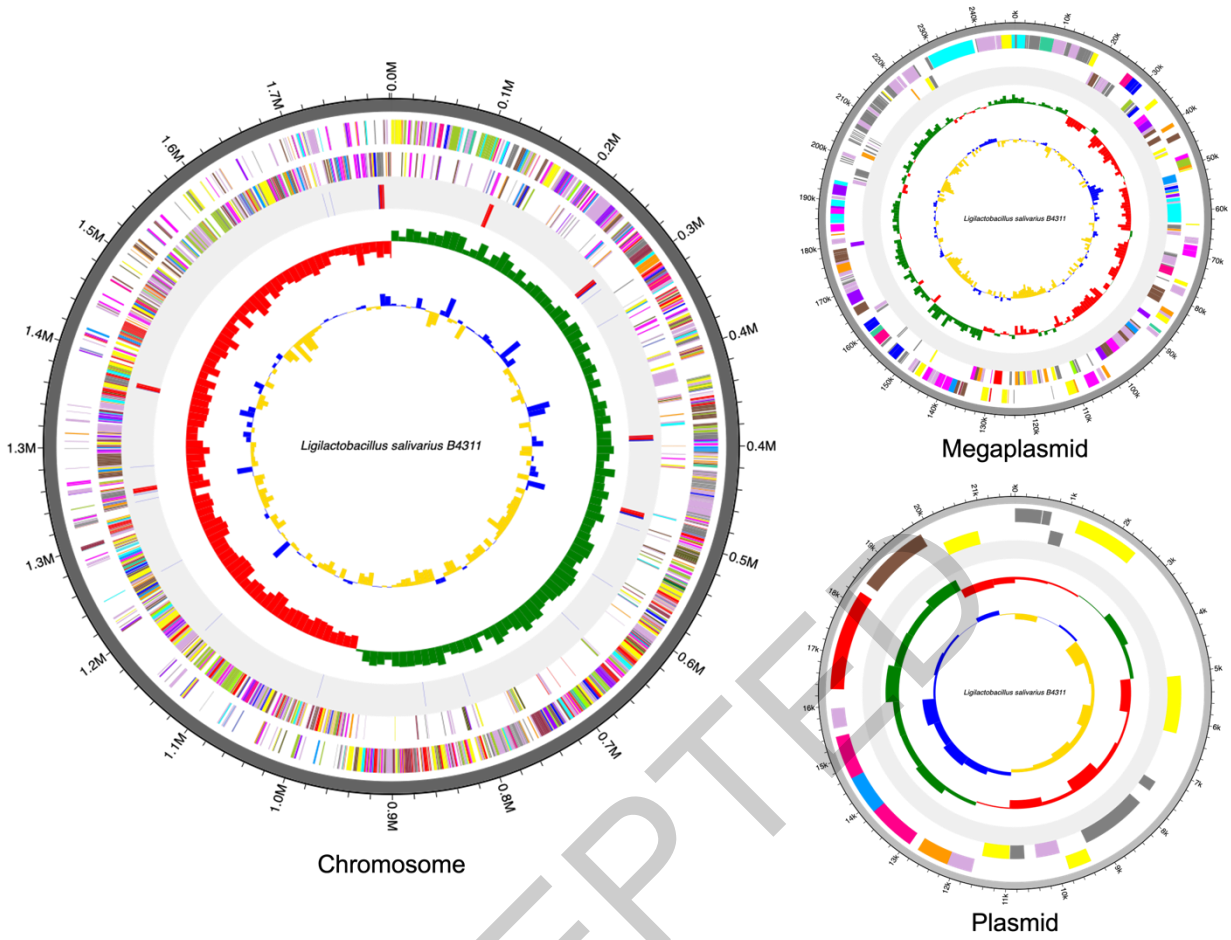

Fig. 1. Circular map of *Ligilactobacillus salivarius* B4311 genome. G, guanine; C, cytosine; CDS, coding sequences. Circles represent the following characteristics from the outermost circle to the center: (1) contig information, (2) coding sequences on forward strand, (3) coding sequences on reverse strand, (4) transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), (5) GC skew, and (6) GC ratio. G, guanine; C, cytosine.

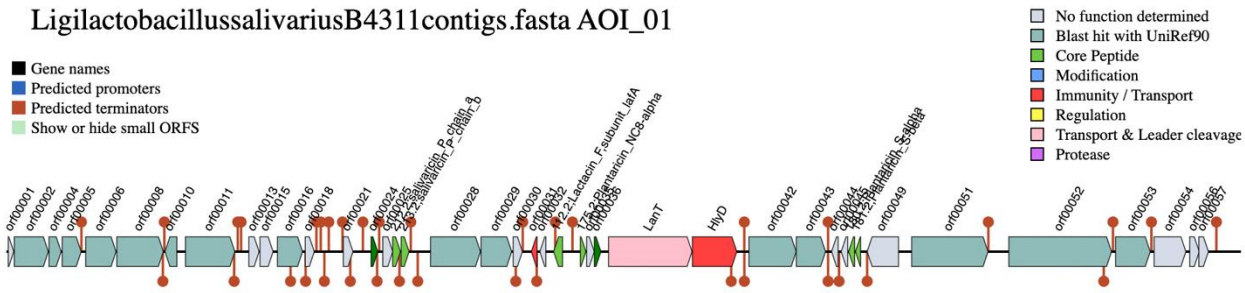

Fig. 2. Predicted bacteriocin gene cluster in *Ligilactobacillus salivarius* B4311genome showing two ORFs for salivaricin P core peptides. ORF, open reading frame.