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Whole genome sequence analysis of *Ligilactobacillus agilis* C7 isolated from pig feces revealed three bacteriocin gene clusters

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No potential conflict of interest relevant to this article was reported.

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Abstract

We here report the whole genome sequence of *Ligilactobacillus agilis* C7 with anti-listerial activity, which was isolated from pig feces. The genome size of *L. agilis* C7 (~ 3.0 Mb) is relatively larger compared with other *L. agilis* strains. *L. agilis* C7 carries three bacteriocin gene clusters encoding garvicin Q, salivaricin A, and Blp family class II bacteriocin. Garvicin Q and salivaricin A are reported to be active against *Listeria monocytogenes* and *Micrococcus luteus*, respectively, as well as against other Gram-positive bacteria. Meanwhile, the bacteriocin encoded in the *blp* cassette was shown to be active against pneumococci, mediating intra-species competition. This report highlights the potential of *L. agilis* C7 for the production of bacteriocins inhibiting pathogenic bacteria.

Keywords: *Ligilactobacillus agilis*, Genome, Bacteriocin, Anti-listerial

ANNOUNCEMENT

Ligilactobacillus agilis, commonly isolated from animals, has been used as a representative species for the study of the motility of lactic acid bacteria, because it has motility features that are uncommon in lactobacilli [1,2]. Very recently, bacterial culture and cell-free supernatant of *L. agilis* 32 isolated from pig manure were reported to inhibit the growth of Enterotoxigenic *Escherichia coli* 10 (ETEC 10), one of the causative agents of post-weaning diarrhea in piglets [3]. In this announcement, we report the genome of *L. agilis* C7, which inhibits *Listeria monocytogenes* (unpublished data), a frequent contaminant of many foods including dairy and meat products, and the three bacteriocin gene clusters encoding garvicin Q, salivaricin A, and a Blp family class II bacteriocin.

L. agilis C7, which showed antibacterial activity against *L. monocytogenes* ATCC 19114 in the agar-well diffusion assay (unpublished data), was isolated from piglet fecal samples. *L. agilis* C7 was cultured in de Man, Rogosa and Sharpe (MRS) broth and streaked on MRS agar. MRS agar plates were incubated at 37℃ for 24 h. Genomic DNA was extracted from the C7 strain using the conventional phenol–chloroform protocol and was sent to Macrogen (Seoul, Korea) for whole-genome sequencing. The PacBio Sequel System (Pacific Biosciences, Menlo Park, CA, USA) and Illumina Platform (Illumina, San Diego, CA, USA) technologies were used to generate long and short sequence reads, respectively. PacBio sequencing generated a total of 2,235,708,853 bases with 237,414 total long reads

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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: Kang DK.
Formal analysis: Mendoza RM, Hwang IC.
Writing - original draft: Yoo JM, Mendoza RM.
Writing - review & editing: Yoo JM, Mendoza RM, Hwang IC, Kang DK.

Ethics approval and consent to participate
This article does not require IRB/IACUC approval because there are no human and animal participants.

(subreads), while Illumina sequencing generated a total of 800,163,663 bases and 5,301,328 paired-end reads after filtering. The Microbial Assembly Application was used for *de novo* assembly of the PacBio reads. Pilon (v1.21) was used to improve the initial assembly by mapping the Illumina reads, generating a more accurate assembly. The genome assembly of *L. agilis* C7 has a total size of 2,961,932 base pair (bp) with four contigs. Quality statistics were generated using QUAST (v5.0.2) and Benchmarking Universal Single-Copy Orthologs (BUSCO) (v5.3.2). QUAST reported the largest contig and N_{50} length of 1,727,998 bp and a guanine + cytosine (G + C) content of 40.15%, while BUSCO reported 124 complete and single-copy BUSCOs. Average Nucleotide Identity, ANIb option (pyani v0.2.10) identified *L. agilis* with > 95% sequence identity to publicly available *L. agilis* genomes.

Annotation of *L. agilis* C7 revealed 93 transfer RNAs, 24 ribosomal RNAs, 1 transfer-messenger RNA, 7 repeat regions, 2946 coding sequences, and 3116 genes (Table 1). BAGEL4 [4] identified two bacteriocin gene clusters encoding the bovicin 255 peptide variant and salivarin A, while AntiSMASH [5] mapped RiPP-like bacteriocin IIc (Fig. 1). The genome size of *L. agilis* C7 is relatively larger (~3.0 Mb) than those of other *L. agilis* strains (~2.0–2.5 Mb) in the NCBI database. Mobile genetic elements such as an integrative and conjugative element (~255 kb) and the 17 prophage regions (7 intact prophages) were also mapped to the genome of *L. agilis* C7 via VRprofile [6].

Blastp analyses of the bovicin 255 peptide variant, salivaricin A, and the RiPP-like bacteriocin IIc revealed 100% identity to garvicin Q family class II bacteriocin from other *L. agilis* strains, 62.5% identity to type A2 lanthipeptide from *Streptococcus pyogenes*, and 51.46% identity to Blp family class II bacteriocin from *S. hyointestinalis*, respectively. Garvicin Q is a subclass IId bacteriocin that was initially isolated from *Lactococcus garvieae* BCC 43578 and has been reported to be active against *L. monocytogenes* and several bacterial species belonging to the genera *Bacillus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, and *Pediococcus* [7]. Meanwhile, salivaricin A, which is active against *Micrococcus luteus* [8], is among the lantibiotics produced by strains of *S. salivarius*, although genetic variants have also been found in *S. pyogenes* [8,9]. Lantibiotics are ribosomally synthesized molecules that are heat-stable and reported to have therapeutic potential in treating infectious diseases. On the other hand, Blp family class II bacteriocin has been shown to mediate intraspecies competition among pneumococci [10].

This report highlights the potential of *L. agilis* C7 for the production of bacteriocin to control *L. monocytogenes* in dairy and meat products. In addition, this paper contributes to the scarce genetic

Table 1. Genome features of *Ligilactobacillus agilis* C7

Features	
Genome size (bp)	2,961,932
No. of contigs	4
N50	1,727,998
GC content (%)	40.15
CDS	2,946
Genes	3116
tRNA	93
Misc RNA	52
rRNA	24
Repeat region	7
tmRNA	1

bp, base pair; GC, guanine + cytosine; CDS, coding sequences.

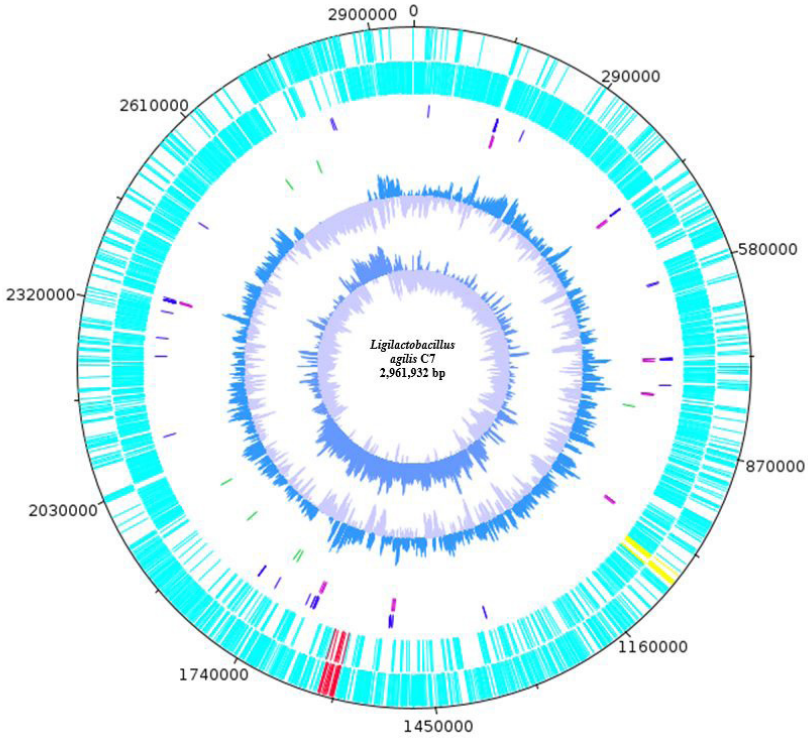

Fig. 1. Genome map of *Ligilactobacillus agilis* C7. Marked features from outside going in: CDS on the forward strand, CDS on the reverse strand (highlighted in yellow and red within the CDS region are the bacteriocin regions for garvicin Q and Blp family class II, and salivaricin A, respectively), tRNAs, rRNAs, repeat regions, GC content, and GC skew. bp, base pair; CDS, coding sequences; GC, guanine + cytosine.

information on *L. agilis*.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER(S)

The genome sequence of *L. agilis* C7 is available in the DDBJ/ENA/GenBank databases under accession no. JAMGEC000000000. The BioSample accession no. is SAMN28229336, and the BioProject accession no. is PRJNA837731.

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