

Note (Genome Announcement)

Draft genome sequence of *Lactobacillus salivarius* KLW001 isolated from a weaning piglet

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이유자돈으로부터 분리한 *Lactobacillus salivarius* KLW001의 유전체 분석

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(Received May 10, 2017; Revised May 12, 2017; Accepted May 17, 2017)

Lactobacillus salivarius KLW001, a species of lactic acid bacteria (LAB), was isolated from a weaning piglet in a swine farm, South Korea, to develop an antimicrobial probiotic strain for piglets. Herein, we report the draft genome sequence of the strain. The genome contains 2,326,706 bp with a G+C content of 33.0% in 166 contigs (≥ 500 bp). From the genome, we found out 4 genes related to antibiotic resistance, 36 genes for phages, 3 genes for bile hydrolysis, and 27 CRISPR spacers.

Keywords: *Lactobacillus salivarius*, antibiotic, genome, piglets, probiotics

Certain species of *Lactobacillus* genera are members of lactic acid bacteria and used as probiotic feed additives for livestock animals (Sung, 2013; Ahn *et al.*, 2016). As one of the species, *Lactobacillus salivarius* is frequently found in the gastrointestinal tracts (GITs) of human and livestock animals, certain strains of the species have been used as probiotics for pigs (Yeo *et al.*, 2016).

We isolated 76 and 208 strains from feces of the weaning

piglets and pig in South Korea, respectively. Their antimicrobial activities were examined against K88 antigen-positive *Escherichia coli* and *Salmonella enterica* serovar Typhimurium strains that were locally isolated from feces of a pig suffered from diarrhea. From the antimicrobial tests, we selected one strain that are strongest among the strains, *L. salivarius* KLW001, from a weaning piglet.

We sequenced the genome of the strain, because we wondered if the strain has any special genomic contents. To purify the genomic DNA, *L. salivarius* KLW001 cells were inoculated into MRS broth from a single colony generated on the MRS agar plate. The cells were incubated at 37°C for 14 h and harvested by centrifugation at 13,000 $\times g$ for 1 min. The cells were washed two times with 1 \times PBS solution. The genomic DNA was extracted using G-spin Total DNA Extraction Kit (Intron Biotechnology) according to the manufacturer's instructions (Kim *et al.*, 2016). We constructed an Illumina sequencing library with ~350-bp inserts for the strain by using Nextera XT DNA Library Preparation Kit (Illumina) according to the manufacturer's recommendations. The library was sequenced on the HiSeq 2500 (Illumina) to obtain 100-bp

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paired-end reads. The Illumina short reads were quality-filtered by removing adapter sequences and low-quality sequences. For the sequence filtering, we used Cutadapt 1.10 (Martin, 2011) and in-house Perl scripts (Kopit *et al.*, 2014). Briefly, when 95% of bases in a sequence were given a quality score over 31 (Illumina 1.8+) and the quality was maintained ≥ 70 bp in length, we used the read for genome assembly. After filtering, we obtained 5,671,822 reads (539,472,474 bp). *De novo* genome assembly was conducted with the filtered reads by using SPAdes 3.9 (Bankevich *et al.*, 2012).

We assembled a draft genome consisting of 2,326,706 bp in 166 contigs (≥ 500 bp) with a G+C content of 33.0%, which are very similar to other released genome sequences of *L. salivarius* in the NCBI Genome Database. The assembled contigs were further processed to be annotated for subsystem classification by using a web-based annotation server, RAST (Overbeek *et al.*, 2014). From the annotation, we obtained 70 tRNA and 2,267 protein-coding sequences, only 46% of which were covered by the subsystem. Four well-known antibiotic resistance genes (*ant61a*, *ermB*, *tetM*, and *tetL*) were identified by analyzing the genome to see if the genome has some of 768 orthologous antibiotic resistance genes, which were previously collected by the antibiotic resistance database (Liu and Pop, 2009). Some of the genes are associated with resistance against antibiotics (streptomycin and tetracycline) that were frequently utilized within the feed in the past. We also identified three genes related to 'Bile hydrolysis' in the genome. Those genes may influence the resistance of the strain to the bile-rich environment in the gut as other gut lactobacilli (Ruiz *et al.*, 2013). The genome has 36 genes associated with 'Phages, Prophages.' We predicted CRISPR sequences by using CRISPRfinder (Grissa *et al.*, 2007) and found out 27 spacers. This draft genome sequence will be helpful to understand the genomic

features of *L. salivarius* strains from piglets when we compare them with others from different origins.

Nucleotide sequence accession number(s)

Lactobacillus salivarius K LW001 strain has been deposited in Korean Agricultural Culture Collection (KACC) under ID KACC 92164P and its draft genome sequence has been deposited at DDBJ/ENA/GenBank under the accession NO. LXZG00000000.

적 요

이유자돈용 생균제 개발을 위해, 본 연구자들은 유산균의 일종인 *Lactobacillus salivarius* K LW001 균주를 대한민국 양돈가에서 사육 중인 이유자돈으로부터 분리하였다. 이 균주는 K88 antigen-positive *Escherichia coli*, *Salmonella enterica* serovar Typhimurium에 대한 항균 활성이 타 균주보다 우수하여, 우리는 이 균주의 유전체를 분석하였다. 유전체 초안 속의 166개 Contig (≥ 500 bp)들에서, G+C content (%)가 33.0%였고, 2,326,706 bp 크기의 염기서열을 확보할 수 있었다. 유전체 초안으로부터 항생제 저항 유전자 4개, Phage 관련 유전자 36개, Bile 대사 유전자 3개, CRISPR Spacer 27개를 확인하였다.

Acknowledgements

This study was supported by a grant from the Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea as part of the multi-ministerial Genome Technology to Business Translation Program (Grant ID:914005-04). Gwi-Deuk Jin and Jun-Yeong Lee were supported by Brain Korea 21 plus program.

Table 1. Genome features of *Lactobacillus salivarius* K LW001

Parameters	Values
Genome size (bp)	2,326,706
No. of contigs (≥ 500 bp)	166
N50 (bp)	43,765
GC content (%)	33.0
No. of tRNA genes	70
No. of protein-coding genes	2,267

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