Note (Genome Announcement)

Draft genome sequence of *Lactobacillus reuteri* KLR3004 from a fattening pig

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비육돈 분변으로부터 분리한 *Lactobacillus reuteri* KLR3004 유산균주의 유전체 분석

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We sequenced the genome of *Lactobacillus reuteri* KLR3004 strain isolated from a fattening pig in South Korea. The sequences were assembled into a draft genome containing 1,996,237 bp with a G+C content of 38.75% and 1,837 predicted protein-coding sequences in 149 contigs.

Keywords: *Lactobacillus reuteri*, antibacterial activity, genome sequencing, probiotic

Lactobacillus spp. are facultative anaerobic and Gram-positive bacteria. Certain *Lactobacillus* sp. have been utilized as probiotics for chickens (Sung, 2013), pigs (Mackenzie *et al*., 2014; Yeo *et al*., 2016) and cattle (Ahn *et al*., 2016). *Lactobacillus reuteri* is one of the frequently used probiotic species for humans and livestock animals.

In this study, we isolated 155 *L. reuteri* strains from pig feces in South Korea. Among the isolates, KLR3004 strain from a fattening pig showed the highest antimicrobial activity against pathogenic bacteria (*Salmonella enterica* serovar Typhimurium

and *K88 antigen-positive Escherichia coli*) that were locally isolated from swine farms in South Korea. The antimicrobial activity plays an important role in the feed industry when *Lactobacillus* strains are used as livestock probiotics (Chiang *et al*., 2015). Thus, we sequenced the genome of the strain with probiotic potential.

To harvest purely cultured cells, a single colony of *L. reuteri* KLR3004 was inoculated into MRS broth and cultivated at 37°C for 24 h without aeration. We harvested bacterial cells from the culture by centrifugation and washed the cells twice with 1× PBS. The genomic DNA was extracted from the cells by using the G-spin Total DNA Extraction Kit (Intron Biotechnology) according to the manufacturer's standard protocol. A DNA library for Illumina sequencing was constructed with ~350 bp inserts by using Nextera XT DNA Library Preparation Kit (Illumina) according to the manufacturer's instructions. The library was then sequenced by using HiSeq 2500 (Illumina) for 100-bp paired-end reads. After the sequencing, adapter sequences were trimmed out with Cutadapt 1.10 (Martin, 2011). The reads were also quality-filtered by using in-house Perl scripts. In

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brief, we selected reads when 95% of bases in a read have ≥31 in quality score (Illumina 1.8+) and ≥70 bp in length. The selected reads (328-fold in depth) were further assembled by using SPAdes 3.9 (Bankevich *et al*., 2012).

We obtained a draft genome of *L. reuteri* KLR3004 containing 149 contigs (contigs ≥500 bp; total length = 1,996,237 bp; G+C contents = 38.75%, Table 1). A total of 1,837 protein coding sequences (CDS) and 66 tRNAs were predicted by an annotation server, Rapid Annotation Using Subsystems Technology (RAST) (Overbeek *et al*., 2014). Genes associated with synthesis of cobalamin were detected in the genome (*cbiD*, *cbiO*, *cbiP*, *hemB*, *hemD*, *sirA*). Such genes are essential to produce bacteriocin called reuterin, that is specific to *L. reuteri*. This strain has 7 genes associated with utilization of arabinose, which were commonly present in *L. reuteri* strains (data not shown). We screened the genome to detect known antibiotic resistance genes, which were collected in the Comprehensive Antibiotic Resistance Database (CARD) (McArthur *et al*., 2013). We found out two antibiotic resistance genes (*lnuA* and *tetW*) responsible for resistance against lincomycin and tetracycline, respectively, which were frequently used for livestock animals before antibiotic ban in Korean feeds (Economou and Gousia, 2015). Three CRISPR spacers were predicted by CRISPRfinder (Grissa *et al*., 2007). This genome will be useful for better understanding of *L. reuteri* strains from pig feces.

Nucleotide sequence accession number

This whole-genome shotgun project has been deposited at GenBank under the accession number MIMT00000000. This study was based on the first version of this genome. *Lactobacillus reuteri* KLR3004 strain has been deposited under ID KACC 92163P.

Table 1. Genome features of *Lactobacillus reuteri* KLR3004

Features	Chromosome
Genome size (bp)	1,996,237
No. of Contigs (≥ 500 bp)	149
GC Content (%)	38.75
No. of tRNA genes	66
No. of Protein-coding genes	1,837

적 요

국내 비육돈에서 분리된 *Lactobacillus reuteri* KLR3004 유산균주의 분석을 실시하였다. 이 유전체 초안은 크기가 1,996,237 bp이며 G+C content (%)는 38.75%이다. 이 초안속의 149개 contig들로부터 1,837개의 단백질 코딩 유전자가 예측되었다.

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