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Short communication

Complete genome sequence of *Bifidobacterium choerinum* FMB-1, a resistant starch-degrading bacterium

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ABSTRACT

The strain *Bifidobacterium choerinum* FMB-1, a bacterium with a strong ability to degrade resistant starch (RS), was isolated from rumen fluids of Korean native cattle (*Bos taurus coreanae*). Degradation experiments revealed that it could degrade approximately 80% of native granular starches within 8 h. Although *B. choerinum* has strong RS degradation abilities, a completed genomic resource has not yet been proposed. Here we present the complete whole genome data of *B. choerinum* FMB-1. It consists of a circular chromosome (2,257,294 bp) and one plasmid (11,012 bp). Genome analysis revealed that at least 11 protein-coding genes were related to α-glucan degradation. The abundance of these genes may affect the efficacy of granular starch degradation. We also found the existence of antimicrobial resistance genes in the genome, which were not reported in other *B. choerinum* genomes. The whole genome information of *B. choerinum* FMB-1 could improve the understanding of the RS degradation mechanism of bovine gut microorganisms.

1. Introduction

Resistant starch (RS) is incompletely digested while passing through the duodenum and small intestine of mammals due to its enzymatic resistance nature (Fuentes-Zaragoza et al., 2011). When incompletely digested, RS reaches the large intestine and is degraded by gut microbiota and used as their energy source. As a result, RS helps to maintain a favorable environment for certain intestinal microflora, including short-chain fatty acid (SCFA)-producing bacteria, which have a considerably positive effect on human health (Bird et al., 2010).

Few studies on RS-degrading microorganisms and related genes or enzymes have been described. At present, two strains, *Ruminococcus bromii* and *B. adolescentis*, have been reported (Ze et al., 2015, 2012). *Bifidobacterium* belonging to the phylum *Actinobacteria* are Gram-positive bacteria with a high GC content and are common microorganisms in the intestines of animals and humans. They are widely used for food, dairy, and health products due to certain probiotic effects, and they show no pathogenicity. *Bifidobacteria* have a beneficial relationship with their host while living in the intestine by promoting health. These beneficial roles include stimulation of the immune response (Lee et al., 1993), protection against virus infection (Saavedra et al., 1994), and prevention of human gastrointestinal disorders, intestinal infection, colonic adenomas, and cancer (Picard et al., 2005). As host-friendly intestinal microorganisms, *Bifidobacterium* species are generally known to utilize polysaccharides such as starch, pullulan, host glycan, and diet-derived polysaccharides (Liu et al., 2015; Ryan et al., 2006). However, not all species of *Bifidobacterium* can degrade RS. To date, only *B. adolescentis* L2-32 has been reported to have an RS-degrading capability when cultured with RS as raw granules (Ze et al., 2012).

Previously, we isolated *B. choerinum* FMB-1 (deposited as KACC 19562) from rumen fluids of Korean native cattle (*Bos taurus coreanae*) and confirmed that it can utilize RS effectively. Here, we report the complete genome sequence of *B. choerinum* FMB-1 and bioinformatic analysis of its gene contents. The genomic analysis combined with biochemical experiments will provide better information to understand the RS degradation mechanism of gut microorganisms.

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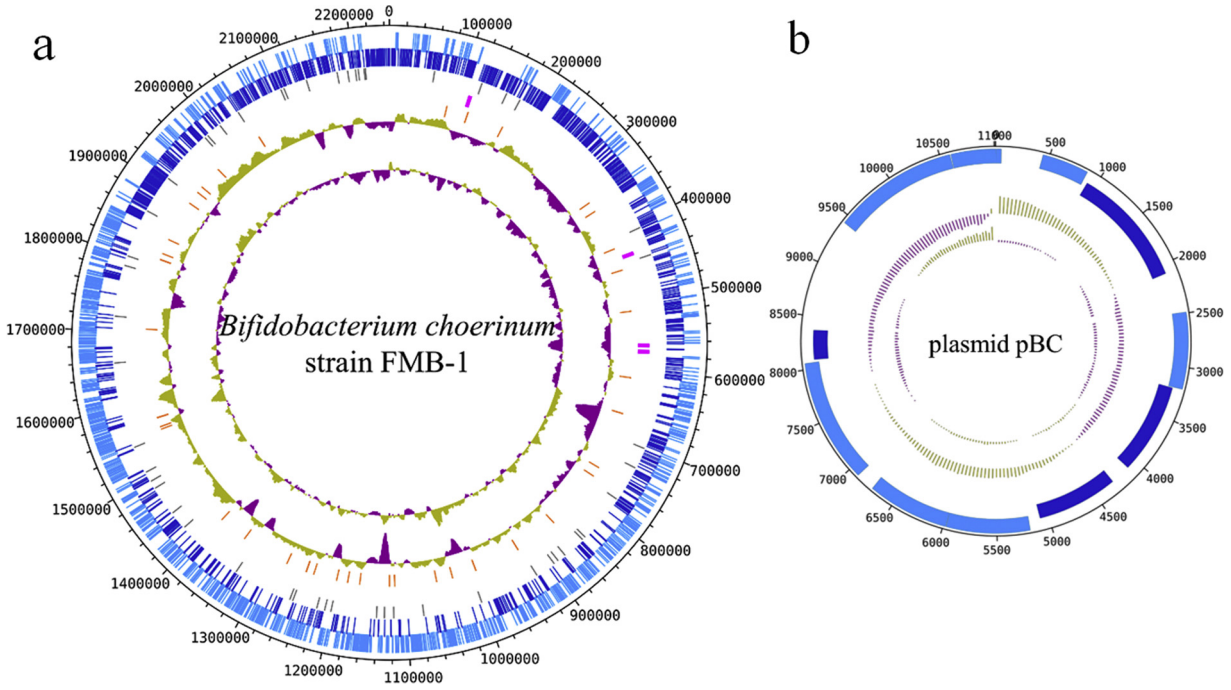

Fig. 1. Circular map of *Bifidobacterium choerinum* FMB-1. a) 2.25 Mbp chromosome, b) 11 Kbp plasmid. Seven tracks were plotted on the map: Track 1 (light blue; outset), forward-strand coding CDS; Track 2 (blue), reverse-strand coding CDS; Track 3 (gray): pseudogenes; Track 4 (light purple), rRNAs; Track 5 (orange), tRNAs; Track 6 (light green and purple), G + C content; and Track 7 (light green and purple): GC skew. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Genome sequencing and assembly

Bifidobacterium choerinum FMB-1 was isolated from rumen fluids of Korean native cattle (*Bos taurus coreanae*) and deposited to Korean Agricultural Culture Collection (KACC, Wanju, Korea) as an accession number of KACC 19562. To analyze the genomic content of a strain *B. choerinum* FMB-1, it was cultivated in MRS medium (Difco Laboratories Inc., Detroit, MI, USA) at 37 °C for 18 h. Genomic DNA was extracted and purified using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The extracted genomic DNA was quantified with a NanoDrop 2000 UV–vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit 2.0 fluorometer (Thermo Fisher Scientific). *B. choerinum* FMB-1 was sequenced with the PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA) sequencing platform.

The sequenced reads were assembled using HGAP 3.0 (Chin et al., 2013) with a 2 Mb expected genome size. Chromosome circularization and correction of the genome start position were performed by utilization of the tool Circlator (Hunt et al., 2015). Determination and annotation of the functional genes were carried out with the NCBI Prokaryotic Genome Annotation Pipeline (PGAAP) (Tatusova et al., 2016).

2.2. Genomic distance between Bifidobacterium genomes

Nineteen genomes belonging to *Bifidobacterium* and *B. choerinum* FMB-1 were used for the estimation of genomic distance. The genomic contents of 19 *Bifidobacteria* (Fig. 2) were downloaded from the NCBI genome database (http://www.ncbi.nlm.nih.gov/genome/). The genomic distance was estimated by a whole genome comparison method known as average nucleotide identity (ANI). The ANI values between genomes were computed using pyani with default options (Tatusova et al., 2016).

2.3. Functional classification

The functional classification of protein-coding genes in FMB-1 was done by the assignment of a Clusters of Orthologous Group (COG) code for each gene. The closest COG gene was obtained by a BLASTP search in the latest COG database with two criteria: 1e-5 of e-value and 50% minimum coverage. Unassigned genes were categorized into 'Not assigned (−)'. For the comparison of functional gene contents of *B. choerinum* strains, previously sequenced three *B. choerinum* genomes, DSM 20434[a], DSM 20434[b], and LMG 10510, were also analyzed by the same method described above.

2.4. Antimicrobial resistance genes

To identify antimicrobial resistance genes, a bioinformatics analysis was carried out using MEGARes (Lakin et al., 2017). A BLASTN search was performed on the protein-coding genes in the MEGARes sequence database. The blast hits were filtered by the following criteria: > 90% identity, > 60% coverage, and 40 bp minimum alignment length.

2.5. Resistant starch utilization

To investigate the degradation of raw starches by *B. choerinum* FMB-1, 2 mL of seed broth was inoculated into 20 mL chopped meat broth containing raw corn starch in a granule form. Starch substrates are composed of 1) 99% amylopectin, 2) 70% amylopectin and 30% amylose, and 3) 30% amylopectin and 70% amylose. A carbon utilization profile of *B. choerinum* FMB-1 was measured at various time points (0, 2, 4, 6, 8, 10, 12, 20, 28, 36, and 44 h) during incubation at 37 °C with gentle mixing.

3. Results and discussion

3.1. The first complete genome of Bifidobacterium choerinum

The genome of *B. choerinum* FMB-1 is composed of two separated circular sequences; one is a 2,246,282 bp chromosome with 65.6% GC content, and the other is an 11,012 bp plasmid with 55.7% GC content (Fig. 1 and Table. 1). A total of 1,857 genes were identified in the FMB-1 genome, including 1,738 protein-coding genes, 68 RNA genes, and 51