


Draft genome sequence of *Brachy bacterium* sp. JHP9 isolated from horse feces in Jeju Island

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제주 말 분변 유래 *Brachy bacterium* sp. JHP9의 유전체 염기서열

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A genus of *Brachy bacterium* known as Gram-positive bacteria were isolated from various environments. In this study, the draft genome sequence of *Brachy bacterium* sp. JHP9, isolated from horse feces, revealed 23 contigs with a genome size of 3,082,019 bp, G + C content of 71.05%, and N50 value of 754,379 bp. Strain JHP9 genome also encodes 2,720 protein-coding genes, 1 rRNA operon (5S, 16S, and 23S), an additional partial 16S rRNA gene, and 51 tRNA genes. Moreover, the strain JHP9 genome carries several genes involved in carbohydrate degradation and lactic acid production. Taken together, the various genomic traits carried by the strain JHP9 make it a potential candidate for lactic acid production.

Keywords: *Brachy bacterium* sp. JHP9, draft genome sequence, genomic features, lactic acid bacteria

The genus *Brachy bacterium* belongs to the family *Derma-bacteriaceae* within the phylum *Actinobacteria*, and a total of 27 *Brachy bacterium* species have been identified in various

ecological niches (<https://lpsn.dsmz.de/genus/brachybacterium>). *Brachy bacterium* species are Gram-positive and isolated from plants, foods including milk products, cheese, salt-fermented seafood, and various environmental samples such as coastal sand, sediment, soil, and seawater (Tak *et al.*, 2018). In addition, a few studies have also reported the isolation of this genus from human samples, such as wound sites, secretion, and microbial flora of the intestine in patients with chronic renal disease (Chang *et al.*, 2016; Park *et al.*, 2016).

Lactic acid bacteria (LAB) is well known as probiotic bacteria and play important roles in food fermentation. Lactic acid produced mainly by LAB can be used in the dairy industry and various industries such as bioplastic (poly-lactic acid) synthesis (Castro-Aguirre *et al.*, 2016). Furthermore, the phage defense systems such as bacteriophage exclusion (BREX) of LAB are considered valuable in industries because it provides the ability to protect strains from phage infection (Hui *et al.*, 2019). This study analyzed the draft genome of strain *Brachy bacterium* sp. JHP9, a candidate of LAB, isolated from horse feces in Jeju Island.

Horse feces were sampled from the grazing land (33°26'

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46.9°N 126°33'50.7"E) and serially diluted to 10^{-4} with PBS (Phosphate Buffered Saline) for isolation of LAB. Then 200 μ l of the diluted sample was spread on *Lactobacilli* MRS agar (MRS, Difco) and followed by incubation at 30°C for 72 h. Several single colonies based on colony morphology and color were selected to inoculate on the new MRS agar. Finally, strain JHP9 was isolated on MRS agar at 30°C.

Genomic DNA of strain JHP9 was extracted according to the manufacturer's protocol using the QIAamp DNA Mini Kit (Qiagen). The extracted genomic DNA was sequenced by Illumina HiSeqX at Macrogen. The sequenced reads were assembled by SPAdes (ver 3.13.0). Then, gene prediction and annotation were performed by NCBI Prokaryotic Genome Annotation Pipeline. Additionally, KEGG (Kanehisa *et al.*, 2016), COG (Galperin *et al.*, 2015) and Pfam (Mistry *et al.*, 2021) annotations were also performed to predict the functions of the putative genes.

Total 13,680,806 reads were sequenced. The assembled genome of strain JHP9 is 3,082,019 bp in size with 71.05% G + C content and 754,379 bp N50 value. The coverage was 85.86 \times . The genome is comprised of 2,720 protein-coding genes, 4 rRNA genes (including a partial 16S rRNA gene), and 51 tRNA genes (Table 1). Identification of this bacterium was performed through 16S rRNA gene amplification, which showed 97.85% similarity with *Brachybacterium nesterenkovi* CIP 104813^T (Gvozdyak *et al.*, 1992).

The genome contained several carbohydrate degradation related proteins such as pullulanase-type alpha-1,6-glucosidase (locus number: Bequi_02280), alpha-amylase (Bequi_00575, Bequi_02325, Bequi_07800 and Bequi_12615), beta-glucosidase

(Bequi_03020 and Bequi_10620), alpha-galactosidase (Bequi_06625), 1,3-beta-glucanase (Bequi_06985 and Bequi_13850), amylo-alpha-1,6-glucosidase (Bequi_07065), 4-alpha-glucanotransferase (Bequi_00145), glycoside hydrolase (GH)3 (Bequi_06250 and Bequi_11035), GH15 (Bequi_13680), GH31 (Bequi_03765), GH 32 (Bequi_04410) and GH65 (Bequi_02085). Prevalence of these proteins indicate the capacity of the strain JHP9 to utilize various classes of carbohydrates.

Lactic acid is produced by lactate dehydrogenase from pyruvate to lactic acid (or lactate) in the process of bacterial carbohydrate fermentation by LAB (Oehmcke-Hecht *et al.*, 2017). The genome of strain JHP9 contains genes coding for the L-lactate dehydrogenase (*ldh*; Bequi_03000) and D-lactate dehydrogenase (*ldhA*; Bequi_04310). Also, lactate racemase was identified on a distinct contig. Therefore, these genes may confer L- and D-lactic acid production ability to the strain JHP9. Furthermore, L- and D-lactic acid can be transformed into inverse isomers of each other (Desguin *et al.*, 2014). This suggests the utilization of the strain JHP9 in various food industry applications.

BREX is considered as a novel bacterial defense system that protects against lytic and temperate phages (Goldfarb *et al.*, 2015). The CRISPR/Cas system is an adaptive immune system of bacteria and archaea, protecting the bacteria from bacteriophages or phages and mobile genetic elements (MGEs) (Barrangou and Marraffini, 2014). In this study, both the BREX system (Bequi_11770 to 11790) and type I-E CRISPR/Cas system (Bequi_14035 to 14070) were observed in the genome of the strain JHP9. These defense strategies carried by this novel strain enable its survival and propagation in various ecosystems, including industrial environments.

Hence, these distinctive genomic features carried by the strain JHP9 make it a potential candidate for LAB and its consequent applications in various industries.

Nucleotide sequence accession and deposit numbers

The draft genome sequence of strain JHP9 was deposited in NCBI GenBank under accession JAKNCJ010000000, and the strain JHP9 was deposited in the KCTC (KCTC 49746) and JCM (JCM 35094).

Table 1. Genomic features of *Brachybacterium* sp. JHP9

Features	Value
Genome size (bp)	3,082,019
Contigs	23
G + C content (%)	71.05
N50 (bp)	754,379
Total genes	2,827
Protein coding genes	2,720
tRNA	51
rRNA (5S, 16S, 23S)	1, 2*, 1
Accession number	JAKNCJ010000000

*One gene is complete, but the other gene is partial.

적 요

Brachybacterium 속의 세균은 그람 양성으로 알려져 있으며, 다양한 환경에서 분리된다. 본 연구에서는 말 분변에서 분리된 *Brachybacterium* sp. JHP9의 유전체가 총 길이 3,082,019 bp의 23개의 contig로 구성되고, 71.05%의 G+C 함량, 754,379 bp의 N50 값을 갖는 것을 확인하였다. 또한 단백질 암호화 유전자 2,720개, rRNA 오페론 (5S, 16S, 23S) 1개, 추가적인 partial 16S rRNA 유전자 1개, tRNA 유전자 51 개로 확인되었다. JHP9 균주는 다양한 탄소분해 및 젖산생성 관련 유전자를 가지고 있으며, 이러한 유전체 특성 분석을 바탕으로 JHP9 균주가 유산균 후보군의 가능성을 확인 하였다.

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Conflict of Interest

The authors have no conflict of interest to report. So-Jeong Kim is Editor of KJM. Dr. Kim was not involved in the review process of this article.

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