


Complete genome sequence of *Pediococcus acidilactici* BB2-4M isolated from cattle faeces

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소 분변에서 분리한 세균 *Pediococcus acidilactici* BB2-4M의 유전체

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Pediococcus acidilacti BB2-4M, isolated from cattle faeces, is potential feed additives to reduce methane gas, improve digestion and inhibit pathogen in the rumen. The genome of strain BB2-4M consists of a circular chromosome (2,040,105 bp) and one plasmid (60,691 bp), with DNA G + C content of 42.0%. The complete genome of strain BB2-4M was identified to have 2,009 genes, including cellulose synthase enzyme and oligopeptide transport proteins. This study will also provide genomic information on oligopeptide transport proteins (*opp* operon) that can be compared with the *Pediococcus acidilacti* DSM 20284^T.

Keywords: *Pediococcus acidilactici*, cattle faeces, genome

Probiotics means microbial dietary supplements, which have beneficial effects on physiology by being involved in the mucosal and systemic immunity of the host (Naidu *et al.*, 1999). Lactic acid bacteria (LAB) are widely used as probiotics in diverse industries such as foods, clinics and livestock (Naidu *et al.*, 1999; Han *et al.*, 2014). In the livestock field, LAB have been studied a lot for reducing methane emissions from ruminants since the LAB are known to act against methanogen growth (Doyle *et al.*, 2019). The genus *Pediococcus* is spherical LAB forming pairs or tetrads and typical homofermentative

LAB which were characterized as Gram-positive, catalase-negative and oxidase-negative (Holzapfel *et al.*, 2006). *Pediococcus* was considered useful in the livestock field. According to Kumar *et al.* (2015), *P. pentosaceus* strain 34 decreased methane production in the *in vitro* experiments. *Pediococcus* spp. have already been studied in various fields as probiotics, which inhibit foodborne bacterial pathogens by producing antimicrobial substances such as pediocin (Motlagh *et al.*, 1994; Rodríguez *et al.*, 2002; Porto *et al.*, 2017). *Pediococcus* is known to live in the rumen of the ruminants (Cobos *et al.*, 2011). We purposed to investigate LAB as feed additives for ruminants, which can reduce methane gas produced by ruminants, help with digestion and inhibit pathogen. Among the LAB strains isolated from cattle feces, *P. acidilactici* BB2-4M reduced methane production and improved feed digestibility *in vitro* fermentation. We conducted genome sequencing and compared the genome sequence of the strain BB2-4M with the type strain *P. acidilactici* DSM 20284^T (GCF_000146325) to invest genomic characteristics.

Pediococcus acidilactici BB2-4M was subcultured on de Man Rogosa and Sharp (MRS) Agar medium at 28°C for 2 days. The genomic DNA of strain BB2-4M was extracted using QIAamp DNA mini kit (Qiagen) by Macrogen, and genome was sequenced by the PacBio Sequel sequencing platform (Pacific Biosciences) and Illumina NovaSeq 6000 (Illumina).

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For PacBio Sequel sequencing, SMRTbell template was used for the generation of a single-SMRT cell, and the data was analyzed via the SMRT Link pipeline version 10.1. NovaSeq reads were used to correct error base pair to improve accuracy. The bacterial genome sequence of the strain BB2-4M was then annotated by the NCBI Prokaryotic Genome Annotation Pipeline software version 6.1 (Tatusova *et al.*, 2016). The amino acid sequences in the FASTA format file were downloaded from NCBI and reannotated using GhostKOALA version 2.2, a homology search tool (Kanehisa *et al.*, 2016). Secondary metabolites including bacteriocin genes were analyzed using antiSMASH (Blin *et al.*, 2021). The orthologous average nucleotide identity (OrthoANI) value between strain BB2-4M and *P. acidilactici* DSM 20284^T was calculated with OrthoANI version 0.5.0 proposed by Lee *et al.* (2016).

Total 94,326 subreads (N50 value, 11,948; genome coverage, 385.2x) were generated in PacBio Sequel and 7,255,050 qualified filtered reads (Q20, 99.0%; mapped reads, 7,254,507; genome coverage, 500.4x) were generated in illumine NovaSeq. Strain BB2-4M consisted of a single circular chromosome (2,040,105 bp) and one plasmid (60,691 bp) with G + C content 42.0% (Table 1). A total of 2,009 genes including 15 rRNAs (5 each of 5S, 16S, and 23S), 56 tRNAs and 4 ncRNAs were predicted. Comparing genomes between strain BB2-4M and *P. acidilactici* DSM 20284^T, an OrthoANI value is 98.2% (99.8% of 16S rRNA gene). Some *P. acidilactici* have a plasmid encoding a cluster of pediocin (Motlagh *et al.*, 1994; Rodríguez *et al.*, 2002). However, strain BB2-4M did not have a plasmid encoding pediocin gene cluster. In addition, oligopeptide transport proteins (*opp* operon; *oppA*, UPU33958; *oppB*, UPU33959;

oppC, UPU33960; *oppD*, UPU33961; *oppF*, UPU33962) were detected which are located in the inner membrane and related to bacterial nutrition, signaling and bacteriocin production (Lee *et al.*, 2004; Kang *et al.*, 2022).

A number of oligopeptides, which are known as quorum sensing signals produced by the LAB, can activate and synthesize bacteriocins. (Gardan *et al.*, 2009; Bu *et al.*, 2021). Although *opp* operon-associated bacteriocin was detected in the genome of strain BB2-4M, no genes coding pediocin and other bacteriocin were found in the results of GhostKOALA and antiSMASH. Therefore, we assumed that the strain BB2-4M has unreported antibiotic genes or was eliminated bacteriocin genes and plasmids encoding pediocin.

This genome sequence can provide a basic understanding of strain BB2-4M as potential feed additives for ruminants.

Nucleotide sequence accession number

Pediococcus acidilactici BB2-4M was deposited in the Korean Agricultural Culture Collection under KACC 22757, and the complete genome sequence has been deposited to GenBank under accession numbers CP096571-CP096572.

적 요

소의 분변에서 분리한 *Pediococcus acidilactici* BB2-4M은 반추동물의 메탄가스 저감, 소화 촉진, 병원균 억제를 위한 잠재적인 사료첨가제이다. 이 균주의 유전체는 원형 염색체(2,040,105 bp)와 한 개의 플라스미드(60,691 bp)로 구성되어 있으며, G + C 함량은 42.0%이다. 이 유전체는 셀룰로오스 생합성 효소 및 올리고펩타이드 전달체를 비롯한 2,009개의 유전자가 확인되었다. 이 연구는 또한 다른 *Pediococcus* 균주들과 비교할 수 있는 올리고펩타이드 수송 단백질(*opp* 오페론)에 대한 유전체 정보를 제공할 것이다.

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Table 1. Genome features of *Pediococcus acidilactici* BB2-4M

Feature	<i>Pediococcus acidilactici</i> BB2-4M
GenBank accession No.	CP096571-CP096572
Genome size (bp)	2,100,796
Chromosome	2,040,105
Plasmid	60,691
G + C content (%)	42.0
Total number of genes	2,009
Protein coding genes (CDS)	1,966
rRNA genes (5S, 16S, 23S)	5, 5, 5
tRNA genes	56
ncRNA genes	4

Conflict to Interest

The researcher claims no conflicts of interest.

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