


Draft genome sequence of *Anaerostipes faecalis* AGMB03513^T, an acetate-, propionate-, and butyrate-producing bacterium isolated from swine faeces

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돼지 분변에서 분리된 아세테이트, 프로피오네이트 및 부티레이트를 생성하는 박테리아인 *Anaerostipes faecalis* AGMB03513^T 균주의 유전체 염기서열 초안

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The genus of *Anaerostipes* is known to have been isolated from the intestines of vertebrates. Likewise, the *A. faecalis* AGMB03513^T was isolated from swine faeces. Whole-genome sequencing of strain AGMB03513^T was performed using a NovaSeq™ 6000 System. The draft genome was consisted of 37 contigs with a total length of 2,544,126 bp and a DNA G + C contents of 37.0%. The genome contained 2,446 protein-coding genes, 8 rRNA, 59 tRNA and 72 pseudogenes. In addition, strain AGMB03513^T contained genes required for acetate, propionate and butyrate synthesis.

Keywords: *Anaerostipes faecalis*, draft genome sequence, swine faeces

Short-chain fatty acids (SCFAs), such as acetate, propionate and butyric acid, are end products produced through the fermentation of gut microbiota (Aho *et al.*, 2021). SCFAs maintained intestinal homeostasis by inducing an anti-inflammatory response and enhancing intestinal barrier function (Parada Venegas *et*

al., 2019; Aho *et al.*, 2021). In addition, SCFAs were used as major energy sources for colon cells and affected cell growth, proliferation and differentiation (Liu, 2015; Parada Venegas *et al.*, 2019). Microbial-produced SCFAs increased IgG concentrations and IgA+ cells, reducing the incidence of diarrhea in weaning pigs and improving immune function (Liu, 2015). Moreover, SCFAs increased pig weight gain by helping lipids and carbohydrates metabolism in the pig intestines and improving feed efficiency (Liu, 2015; Zhou *et al.*, 2021).

The genus *Anaerostipes* was firstly proposed as valid name by Schwartz *et al.* (2002), and belonged to the family *Lachnospiraceae* within the phylum *Firmicutes*. Currently, the genus *Anaerostipes* consisted of 8 valid species: *A. caccae*, *A. butyraticus*, *A. hadrus*, *A. rhamnosivorans*, *A. hominis*, *A. faecis*, *A. faecalis*, and *A. amylophilus* (<https://lpsn.dsmz.de/genus/anaerostipes>). The members of the genus *Anaerostipes* were isolated from human intestine or faeces, broiler chicken contents, swine faeces and mouse faeces (Choi *et al.*, 2021; Hitch *et al.*, 2021; Lee *et al.*, 2021; Forster *et al.*, 2022). The genus *Anaerostipes* was strictly anaerobic, rod-shape, non-motile,

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gram-variable bacteria, which produced butyrate or had related genes (Choi *et al.*, 2021; Hitch *et al.*, 2021; Lee *et al.*, 2021; Forster *et al.*, 2022). Here, we describe the draft genome sequence and annotation of *Anaerostipes faecalis* AGMB03513^T, which we have reported as a novel species.

The strain was cultured using RCM medium (MB cell) in anaerobic chamber (Coy Laboratory Products) containing 90% N₂, 5% H₂ and 5% CO₂. Genomic DNA (gDNA) was extracted using a phenol:chloroform:isoamyl alcohol method (Wilson *et al.*, 1990). The extracted gDNA was measured for quantity and quality with PicoGreen. Then, the extracted gDNA sheared using Covaris LE220 (Covaris). A DNA library was prepared using a TruSeq Nano DNA kit (Illumina). Validation of final product was analyzed using an Agilent 2100 Bioanalyzer (Agilent Technologies). Whole-genome sequencing was performed using a NovaSeq™ 6000 System (Illumina) with paired-end read lengths of 151 bp. *De novo* assembly was performed with the SPAdes version 3.13.0 (Bankevich *et al.*, 2012). Potential contamination of genomic assembly was confirmed by ContEst16S (Lee *et al.*, 2017) and quality was checked by CheckM (Parks *et al.*, 2015). The coding DNA sequences (CDSs) and tRNAs were predicted using Prodigal and tRNAscan-SE, respectively. Genome annotation and analysis were performed using Prodigal, NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova *et al.*, 2016) and KEGG (Kanehisa *et al.*, 2014).

Table 1. General features of *Anaerostipes faecalis* AGMB03513^T

Property	Value
Genome assembly	
Assemble method	SPAdes v. 3.13.0
Genome coverage	1306.6×
Genome features	
Genome size (bp)	2,544,126
DNA G + C content (%)	37.0
No. of contigs	37
Total genes	2,589
Protein-coding genes	2,446
Complete rRNA genes	1 (5S)
Partial rRNA genes	4, 3 (16S, 23S)
tRNA genes	59
Other RNA	4
Pseudogenes	72
GenBank Accession No.	JABRXE000000000

The draft genome of *A. faecalis* AGMB03513^T was consisted of 37 contigs with 1306.6× coverage. The length of genome was 2,544,126 bp (N50 value, 232,061 bp) and the DNA G + C content was 37.0%. The NCBI PGAP annotation showed that the genome contains 2,446 protein-coding genes, 8 rRNA, 59 tRNA and 72 pseudogenes (Table 1).

We identified several producible SCFA synthesis pathways in the genome of strain AGMB03513^T. Among them, previous studies showed strain AGMB03513^T to produces butyrate, acetate and propionate (Choi *et al.*, 2021). First, the genome contained pyruvate ferredoxin oxidoreductase (KTB61_RS05235), acetyl-CoA C-acetyltransferase (KTB61_RS02085), 3-hydroxybutyryl-CoA dehydrogenase (KTB61_RS02095), enoyl-CoA hydratase (KTB61_RS02090) and enoyl-[acyl-carrier protein] reductase (KTB61_RS02260) genes for synthesizing butyrate from pyruvate. Strain AGMB03513^T had pyruvate ferredoxin oxidoreductase (KTB61_RS05235), phosphate acetyltransferase (KTB61_RS01375) and acetate kinase (KTB61_RS01380) to produce acetate from pyruvate. Finally, propionate can be synthesized from pyruvate via two pathways. One pathway involved a phosphate acetyltransferase (KTB61_RS01375) and acetate kinase (KTB61_RS01380) genes, and the other involved a l-lactate dehydrogenase (KTB61_RS02120, KTB61_RS02185, KTB61_RS06530) and acetate kinase (KTB61_RS01380) genes.

We performed whole-genome sequencing to understand how strain *A. faecalis* AGMB03513^T produces acetate, propionate and butyrate. These studies suggest that it will help to understand the physiological function of strain AGMB03513^T, which synthesizes acetate, propionate and butyrate in the swine gut environment.

Nucleotide sequence accession number

Anaerostipes faecalis AGMB03513^T has been deposited in the Korean Collection for Type Cultures under accession number KCTC 25020^T. The GenBank/EMBL/DDBJ accession number for the genome sequence of *A. faecalis* AGMB03513^T is JABRXE000000000.

적 요

Anaerostipes 속은 척추동물 내장의 내용물에서 분리된 것으로 알려져 있다. *Anaerostipes faecalis* AGMB03513^T도 돼지 분변에서 분리되었으며, AGMB03513^T의 전장 유전체 시퀀싱은 NovaSeq™ 6000 시스템을 이용하여 수행했다. 유전체 초안은 총 길이 2,544,126 bp와 37.0%의 DNA G + C 함량을 갖는 37개의 contig들로 구성되어 있고, 유전체에는 2,446개의 단백질 코딩 유전자, 8개의 rRNA, 59개의 tRNA 그리고 72개의 위유전자를 포함하고 있다. 또한, 균주 AGMB03513^T는 아세테이트, 프로피오네이트 및 부틸레이트 합성에 필요한 유전자를 포함한다.

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Conflict of Interest

The authors have no conflict of interest to report.

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