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Complete Genome Sequence of *Bacillus* sp. Strain hwrm1 Isolated from the Rumen of Korean Native Cattle Hanwoo

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***Bacillus* sp. strain hwrm1, isolated from the rumen of Korean native cattle Hanwoo (*Bos taurus coreanae*), exhibit phytic acid-degrading, cellulose-degrading and antimicrobial activities. The complete genome of strain hmrw1 consists of a circular chromosome in the size of 4,141,581 bp with a GC content of 46.1% GC contents. The genome contains 3,976 genes, including genes encoding a phytase, various glycoside hydrolases, and antibiotic peptides, aligning with its activities. The genomic information is crucial for further characterization and application of the strain as a probiotic.**

Keywords: ***Bacillus*, Hanwoo steer, rumen**

Phytic acid (myo-inositol hexakisphosphate; IP6) is a storage form of phosphorus found in many plant tissues, including legumes, bran, seeds, and nuts [1] . In livestock, phytic acid acts as an anti-nutritional factor by inhibiting the absorption of essential minerals such as calcium, iron, and zinc [2]. To counter this, enzymes called phytases are incorporated into livestock feed to degrade phytic acid, thereby enhancing feed absorption efficiency [3]. The global animal feed enzyme market is projected to undergo a compound annual growth rate (CAGR) of 6.5% from 2023 to 2032, culminating in a market valuation of approximately $3.3 billion [4]. Previous study has identified various microbes that produce phytase [1]. In this study, a phytic acid-degrading bacterium was isolated from the rumen of Korean native cattle Hanwoo (*Bos taurus coreanae*). Additionally, the strain exhibited cellulose-degrading and antimicrobial activities, indicating its potential as a probiotic for livestock. To further understand these activities, the complete genome sequence of the strain was analyzed.

A bacterial strain exhibiting phytic acid-degrading activity was isolated from the rumen of a 16-month-old Hanwoo steer. According to its 16S rRNA gene sequence, the strain was designated to *Bacillus* sp. strain hwrm1. The study was approved by the Ethics Committee of the National Institute of Animal Science (approval no: NIAS2023-0605). To evaluate phytic acid-degrading activity, the strain was cultured on phytic acid agar medium (20.0 glucose, 4.0 sodium phytate, 5.0 NH_4NO_3, 2.0 $CaCl_2$, 0.5 KCl, 0.5 $MgSO_4 \cdot 7H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$, 0.01 $MnSO_4 \cdot H_2O$, and 20.0 agar in g/l). The presence of a clear zone indicated phytic acid degradation. For a more accurate assessment, a phytic acid-supplemented

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medium (+phy medium) was prepared as previously described [5]. The strain was cultured in a liquid +phy medium in triplicate, and residual phytic acid levels were measured using the Phytic Acid Assay Kit (Megazyme, Ireland). The amount of phytic acid decreased by 45.12% after 12 h incubation at 37℃. In addition, the strain exhibited cellulose-degrading activity when cultured in a CMC-Trypan medium (10.0 tryptone, 5.0 yeast extract, 10 NaCl, 5.0 carboxymethyl cellulose (CMC), 0.02 trypan blue, and 15.0 agar in g/l). The filter-sterilized culture supernatant of the strain also displayed antimicrobial activity, inhibiting the growth of pathogenic bacteria, *Listeria monocytogensis* ATCC 19111 and *Salmonella enterica* ATCC 4931.

Genomic DNA of the strain was extracted using the Qiagen MagAttract High Molecular Weight DNA kit (Qiagen, Germany). The genome was sequenced using the PacBio Sequel IIe sequencing platform (Pacific Biosciences, USA) and Illumina NovaSeq 6000 (Illumina, USA). For PacBio sequencing, the SMRTbell template was employed to generate a single-SMRT cell, and assembly was conducted using the SMRT Link pipeline (version 11.0). NovaSeq reads were used to correct erroneous base pairs using the Pilon (version 1.21). Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline software (PGAP, version 6.5) and Prokka (version 1.14.6) [6]. The genome of *Bacillus* sp. hwrm1 was assembled into a single circular chromosome with a length of 4,141,581 bp, a GC content of 46.1%, and a coverage of 318× with no plasmid detected. The genome includes 3,976 protein-coding genes (CDS), 27 rRNAs, 86 tRNAs, and 5 ncRNAs.

A phylogenomic tree of *Bacillus* sp. strain hwrm1 was constructed using reference genomes of 122 species within the genus *Bacillus,* which were obtained from the NCBI database as of July 2024. Orthologous gene fami-

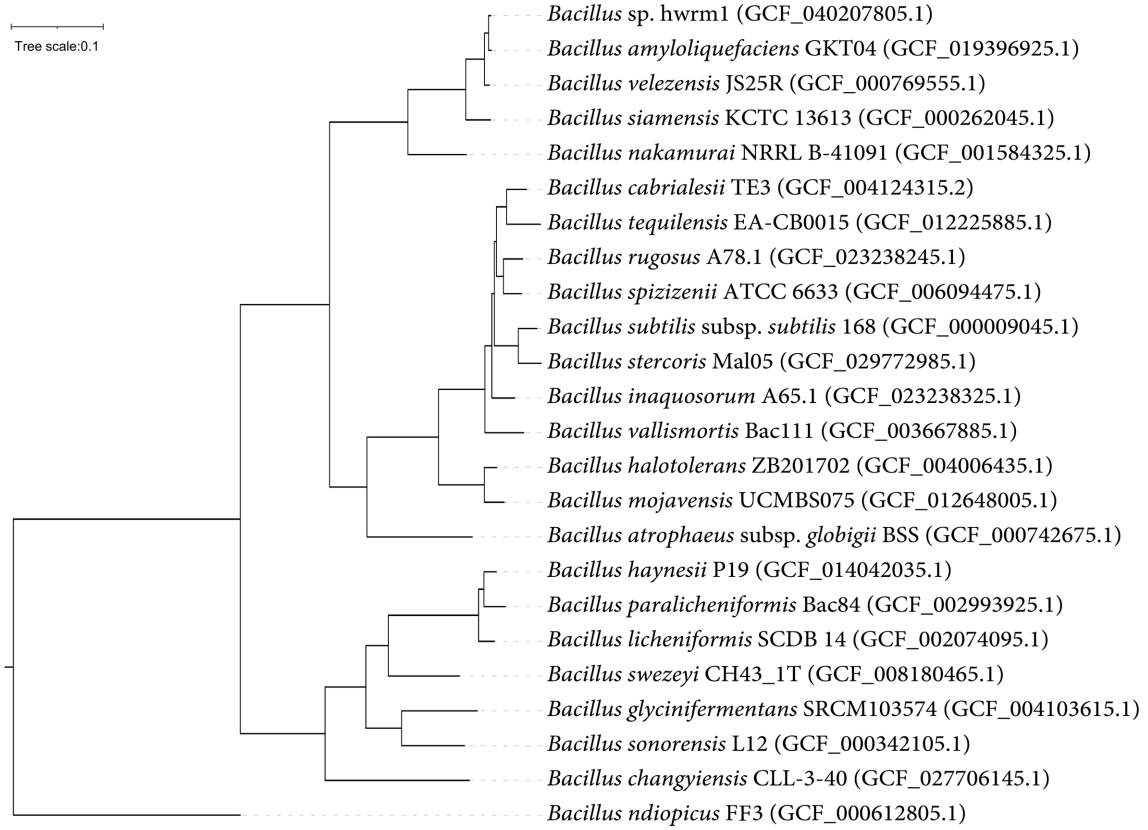

Fig. 1. Phylogenomic tree of a phylogenetic branch of the genus *Bacillus* including strain hwrm1. The tree was constructed using the maximum-likelihood method, based on a set of 43 core genes obtained from genomes of 22 reference genomes within the branch and the genome of strain hwrm1. *B. ndiopicus* strain FF3 was used as an outgroup. NCBI RefSeq assembly accession numbers for all genomes are provided in brackets.

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lies of these genomes were obtained and clustered using the PIRATE (version 1.0.5). The clusters derived at a 95% identity cutoff were aligned using the MAFFT (version v7.520). The tree was constructed based on these clusters using FastTree and visualized using iTOL v6 tools. A distinguished phylogenetic branch of the tree including strain hwrm1 and 22 reference strains is shown in Fig. 1. *B. ndiopicus* strain FF3 from another phylogenetic branch was used as an outgroup. The strain was clustered together with *B. amyloliquefaciens* strain GKT04, *B. velezensis* strain JS25R, and *B. siamensis* strain KCTC 13613 (Fig. 1). The average nucleotide identity (ANI) values between strain hwrm1 and these strains were 99.5%, 98.0%, and 94.3%, respectively.

In the genome of strain hwrm1, functional genes related to its defined activities were investigated. For phytic acid degradation, a gene encoding 3-phytase (locus tag No. ABK447_1058 in the GenBank accession No. CP157943) was identified. The protein sequence was found to be identical to previously annotated phytase proteins in *B. amyloliquefacines* (GenBank accession numbers MBE7959593.1 and MBG9463988.1) and *B. velezensis* (GenBank accession numbers ASP26575.1, ATO00356.1, and AZI47312.1). To understand its cellulose-degrading activity, glycoside hydrolases (GHs) in the genome were identified using the dbCAN3 tool [7]. A total of 43 GHs were found, among which 17 GHs are suggested to be secreted enzymes based on the the presence of signal sequence predicted by SignalP tool within the dbCAN3 (Table 1). Among these secreted GHs, families related to cellulase activity such as GH5 and GH26, were included, corresponding with its extracellular cellulase activity. Additionally, the genome contains genes responsible for the production of antibiotic peptides. As reported in the genomes of in *B. velezensis* and *B. amyloliquefaciens*, the complete biosynthetic gene cluster for dipeptide antibiotic bacilysin (*bacABCDE*) was conserved in the genome [8]. The complete surfactin gene cluster (*srfAA*, *srfAB*, *srfAC*, and *srfAD*) was also found in the genome [9].

The functional characterization and complete genome sequence of *Bacillus* sp. strain hwrm1 demonstrates potential as a probiotic for livestock based on its benefi-

Table 1. Extracellular glycoside hydrolases (GHs) in the genome of *Bacillus* sp. strain.

Glycoside hydrolase family*	GenBank locus tag**	Annotation***	Signal peptide cleavage*
GH3	ABK447_19800	β-Hexosaminidase	21/22
GH5	ABK447_11355	Endoglucanase	31/31
GH11	ABK447_02180	Endo-1,4-β-xylanase A	29/30
GH13	ABK447_19130	α-Amylase	34/35
GH16	ABK447_00950	β-glucanase	30/31
GH26	ABK447_01085	Mannan endo-1,4-β-mannosidase	25/26
GH30	ABK447_11335	Glucuronoxylanase XynC	34/35
	ABK447_10730	Hypothetical protein	31/32
GH43	ABK447_00830	Extracellular endo-α-(1→5)-L-arabinanase	31/32
	ABK447_06175	Extracellular endo-α-(1→5)-L-arabinanase	27/28
	ABK447_11330	Arabinoxylan arabinofuranohydrolase	26/27
GH46	ABK447_04225	Chitosanase	37/38
GH53	ABK447_14505	Arabinogalactan endo-β-1,4-galactanase	35/36
GH68	ABK447_00240	Levansucrase	30/31
GH73	ABK447_02610	β-*N*-acetylglucosaminidase	28/29
GH126	ABK447_18510	Hypothetical protein	25/26
GH171	ABK447_19805	Hypothetical protein	24/25

*GH family and signal sequence prediction were conducted using the dbCAN3 tool.
**From the NCBI GenBank accession number CP157943.1.
***Annotation was performed using the Prokka (version 1.14.6) annotation pipeline.

cial activities as well as its origin. This genome information is vital for further functional characterization and application of the strain.

Culture Collection Deposit and Nucleotide Sequence Accession Number

Bacillus sp. strain hwrm1 was deposited in the Korean Agricultural Culture Collection under the accession number KACC 81299BP. The complete genome sequence of the strain has been deposited in the NCBI GenBank under the accession number CP157943.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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