


Complete genome sequence of *Bacillus inaquosorum* 1HC-NA assembled from the Oxford Nanopore sequencing data

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옥스포드 나노포어 시퀀싱 데이터로 조립한 *Bacillus inaquosorum* 1HC-NA의 전장 유전체

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Bacillus inaquosorum 1HC-NA is isolated from the soybean rhizosphere soil in Republic of Korea. The complete genome sequence of *B. inaquosorum* 1HC-NA was obtained through Oxford Nanopore sequencing. The total genome length of this strain is 4,240,317 bp with 44.0% of GC content. This strain contains 3,293 protein-coding genes, 30 rRNA genes, 86 tRNA genes and 5 ncRNA genes. Furthermore, Rapid Annotation using Subsystem Technology (RAST) predicted a total of 332 subsystems of the strain, including amino acids and derivatives and carbohydrates subsystem.

Keywords: *Bacillus inaquosorum* 1HC-NA, complete genome, long-read sequencing, Oxford Nanopore sequencing

Ubiquitous in nature, *Bacillus* genera interact symbiotically with various microbes and hosts (Jo *et al.*, 2020; Wilkes *et al.*, 2020; Torres-Sánchez *et al.*, 2021). Recently, *Bacillus* spp. have been suggested as the biocontrol agents to promote the eubiosis of the soil microbiome in the agricultural industry (Jo *et al.*, 2020; Wilkes *et al.*, 2020). Therefore, we isolated *Bacillus inaquosorum* 1HC-NA from the soybean rhizosphere soil and identified its genome sequence. The soybean rhizosphere soil

was collected from Sangju-si, Gyeongsangbuk-do, Republic of Korea. From this, the bacterial isolation was conducted with the following methods: 1 g of soil attached to the soybean roots was taken, suspended in saline, 100 µl of suspension plated on nutrient agar (NA; Difco). *Bacillus inaquosorum* 1HC-NA is available in Korean Collection for Type Cultures under the accession number KCTC 13372BP.

The genomic DNA extraction was performed using Wizard[®] Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. Qubit 3.0 fluorometer (Thermo Fisher Scientific) and Nanodrop One Spectrophotometer (Thermo Fisher Scientific) were used for DNA quantification and qualification, respectively. In order to prepare the Nanopore sequencing library, Ligation Sequencing Kit SQK-LSK109 (Oxford Nanopore Technologies [ONT]) and NEBNext[®] Companion Module for Oxford Nanopore Technologies[®] Ligation Sequencing Kit (NEB) were used. The prepared sequencing library was loaded into the FLO-MIN111 (R10.3, ONT). The sequencing was performed during 72 h and Guppy (v.4.4.1) was used for basecalling (Wick *et al.*, 2019). A total of 501,492,938 reads with Phred score higher than 7 was generated with 20,700 bp of N₅₀. *De novo* assembly of sequences was performed using Flye (v.2.9) with default parameters, excepting

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Table 1. Genome features of *B. inaquosorum* 1HC-NA genome sequence

Genome feature	Value
Genome length (bp)	4,240,317
GC content (%)	44.0
Total number of genes	4,359
Number of protein-coding genes	3,293
Total number of RNA genes	121
rRNA genes (5S, 16S, 23S)	10, 10, 10
tRNA genes	86
ncRNA genes	5
Pseudo genes	945

size option (option: -nano-raw -genome-size 4.0) (Kolmogorov *et al.*, 2019).

The complete genome of *B. inaquosorum* 1HC-NA comprises one chromosome with 4,240,317 bp of genome length and 44.0% of GC content. Furthermore, the genome was annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova *et al.*, 2016) and the Rapid Annotations using Subsystem Technology (RAST) server (Aziz *et al.*, 2008). *Bacillus inaquosorum* 1HC-NA contains 4,359 protein-coding genes, 30 rRNAs, 86 tRNAs, 5 ncRNAs, and 945 pseudo genes (Table 1). The RAST server predicted 332 of classified subsystems with 30% of coverage. Amino acids and derivatives related features (372 genes), carbohydrates (324 genes), protein metabolism (248 genes), cofactors, vitamins, prosthetic groups, pigments subsystem (181 genes), cell wall and capsule

(112 genes) and nucleosides and nucleotides (112 genes) were the predominant subsystems in the strain. Furthermore, the strain 1HC-NA has the ability to antimicrobial activities, such as bacilysin and antimicrobial peptide biosynthesis. The genome contains *bacA*, *bacB*, *bacC*, and *bacG*, which encode the bacilysin biosynthesis protein (Table 2). Additionally, antimicrobial peptides, including sublancin and plipastatin, can be produced through the *sunA*, *sunS*, and *ppsA* in the strain 1HC-NA. These results suggest that *B. inaquosorum* 1HC-NA is a potent biocontrol bacterium for agricultural purposes.

Nucleotide sequence accession numbers

The complete genome sequence data of *B. inaquosorum* 1HC-NA was deposited in GenBank with accession number (CP101611.1).

적 요

한국의 콩 근권 토양으로부터 *Bacillus inaquosorum* 1HC-NA를 분리하였다. Oxford Nanopore sequencing을 통해 *B. inaquosorum* 1HC-NA의 전장 유전체가 확보되었다. 전장 유전체는 총 44.0%의 GC 함량을 포함하며 4,240,317 bp의 길이를 가졌다. 이 균주는 3,293개의 단백질 코딩 유전자와 30개의 rRNA, 86개의 tRNA, 5개의 ncRNA를 보유하고 있다. 또한, Rapid Annotation using Subsystem Technology (RAST)는 아

Table 2. The list of biocontrol related genes in the genome of *B. inaquosorum* 1HC-NA

Gene	Size (bp)	Protein	Locus-tag
<i>bacA</i>	615	Biosynthesis of the antibiotic bacilysin	NM058_02385
<i>bacB</i>	708	Biosynthesis of the antibiotic bacilysin	NM058_02390
<i>bacC</i>	762	Biosynthesis of the antibiotic bacilysin	NM058_02395
<i>bacE</i>	1185	Self-protection to bacilysin	NM058_02405
<i>bacG</i>	780	Biosynthesis of the antibiotic bacilysin	NM058_02415
<i>albG</i>	702	Antilisterial bacteriocin (subtilosin) production	NM058_02550
<i>albD</i>	1,311	Export of antilisterial bacteriocin (subtilosin)	NM058_02565
<i>albB</i>	162	Antilisterial bacteriocin (subtilosin) production	NM058_02575
<i>albA</i>	1,347	Antilisterial bacteriocin (subtilosin) production	NM058_02580
<i>sboA</i>	132	Antimicrobial peptide	NM058_02590
<i>bpsB</i>	507	Polyketide synthesis	NM058_10865
<i>sunA</i>	171	Exported antimicrobial peptide	NM058_11590
<i>sunS</i>	1,269	Biosynthesis of the antimicrobial peptide sublancin	NM058_11605
<i>ppsA</i>	2,613	Production of the antibacterial compound plipastatin	NM058_11755

미노산 및 탄수화물 subsystem을 포함하여 균주의 총 332개의 subsystem을 예측하였다.

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Conflict of Interest

The authors have no conflict of interest to report.

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