

Genome Reports

Complete Genome Sequence of *Bacillus subtilis* NIB353 Isolated from Nuruk

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Thermotolerant *Bacillus subtilis* NIB353 was isolated from Nuruk, a traditional Korean fermentation starter. The complete *B. subtilis* NIB353 genome sequence was obtained using MinION and Illumina (MiSeq) platforms. The *B. subtilis* NIB353 genome sequence was 4,247,447 bp with a GC content of 43%. The *B. subtilis* NIB353 strain exhibited orthologous average nucleotide identity values of 98.39% and 98.38% with *B. subtilis* 168 and *B. subtilis* ATCC6051a, respectively. The genome has been deposited in GenBank under the accession number NZ_CP089148.1.

Keywords: *Bacillus subtilis*, complete genome sequence, whole-genome analysis, fermentation starter culture, Nuruk

Bacillus subtilis is a culture starter commonly used in the preparation of traditional fermented foods, such as cheonggukjang, natto, and kinema [1]. Due to its highly efficient protein secretion system and adaptive metabolism, *B. subtilis* is widely used in industrial applications [2]. In the present study, we describe *B. subtilis* NIB353, which can grow at 57°C and was isolated from Nuruk, a traditional Korean fermentation starter.

B. subtilis NIB353 was inoculated in nutrient broth and incubated at 37°C with agitation for 24 h. Following incubation, bacterial cells were harvested by centrifugation at 20,000 × *g* for 10 min. Subsequently, genomic DNA was extracted using the ExiPrep™ Plus Bacteria Genomic DNA Kit (Bioneer, Korea), according to the manufacturer's instructions. The complete *B. subtilis* NIB353 genome was sequenced using the MinION and Illumina (MiSeq) platforms. The read set was assembled *de novo* into one contig sequence using Unicycler

(v0.4.8). *B. subtilis* NIB353 genome annotation was performed using rapid prokaryotic genome annotation (Prokka v1.14.6) [3] and rapid annotation using subsystems technology (RAST) [4]. Average nucleotide identity (ANI) values among the *B. subtilis* NIB353 genome and genomes of closely related species (*B. subtilis*, *Bacillus halotolerans*, *Bacillus mojavensis*, *Bacillus amyloliquefaciens*, *Bacillus velezensis*, *Bacillus licheniformis*, and *Bacillus paralicheniformis*) were calculated using the Orthologous Average Nucleotide Identity Software Tool (OAT, v0.93.1) [5].

The genomic features of *B. subtilis* NIB353 and the other *Bacillus* species are highlighted in Table 1. The *B. subtilis* NIB353 genome, which consists of one contig, was 4,247,447 bp long with a GC content of 43%. The genome size and GC content are in alignment with those of *Bacillus* species. In addition, the *B. subtilis* NIB353 genome was predicted to contain 4,350 coding sequences, 30 rRNAs, and 87 tRNA genes. Using RAST-based annotation, 337 functional subsystems were revealed (Table 2).

The *B. subtilis* NIB353 strain exhibited orthologous

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Table 1. Comparison of genomes between *B. subtilis* NIB353 and several other *Bacillus* species.

Strain	GC content (%)	Contig N50 (bp)	Predicted CDS	rRNAs	tRNAs	GenBank accession NO.	Reference
<i>B. subtilis</i> NIB353	43.0	4,247,447	4,350	30	87	NZ_CP089148.1	This study
<i>B. subtilis</i> ATCC6051a	43.5	4,215,633	4,244	30	86	NZ_CP011115.1	[6]
<i>B. subtilis</i> 168	43.5	4,215,606	4,237	30	88	NC_000964.3	[7]
<i>B. halotolerans</i> ZB201702	43.8	4,154,245	4,023	30	88	NZ_CP029364.1	[8]
<i>B. mojavensis</i> UCMB5075	43.8	4,031,121	3,950	30	87	NZ_CP051464.1	-
<i>B. amyloliquefaciens</i> IT-45	46.6	3,928,857	3,725	30	95	NC_020272.1	[9]
<i>B. velezensis</i> JS25R	46.4	4,006,002	3,761	21	81	NZ_CP009679.1	-
<i>B. licheniformis</i> SCEB14	46.3	4,136,986	4,070	24	81	NZ_CP014842	-
<i>B. paralicheniformis</i> Bac84	45.8	4,376,831	4,237	24	81	NZ_CP023665.1	[10]

Abbreviations: B., *Bacillus*; CDS, coding sequence; rRNA, ribosomal RNA; tRNA, transfer RNA

Table 2. Overview of the subsystem categories among *B. subtilis* NIB353 and closely related strains (*B. subtilis* ATCC6051a and *B. subtilis* 168).

	<i>B. subtilis</i> NIB353	<i>B. subtilis</i> ATCC6051a	<i>B. subtilis</i> 168
Number of subsystems	337	334	334
Subsystem coverage			
In subsystem	1,205 (27%)	1,180 (27%)	1,179 (27%)
Hypothetical	1,143	1,119	1,118
Non-hypothetical	62	61	61
Non in subsystem	3,429 (73%)	3,226 (73%)	3,230 (73%)
Hypothetical	1,611	1,652	1,653
Non-hypothetical	1,818	1,574	1,577
Subsystem category distribution			
Cofactors, vitamins, prosthetic groups, Pigments	143	145	145
Cell wall and capsule	92	83	84
Virulence, disease and defense	35	39	39
Potassium metabolism	3	3	3
Photosynthesis	0	0	0
Miscellaneous	25	25	25
Phages, prophages, transposable elements, plasmids	26	7	7
Membrane transport	44	42	42
Iron acquisition and metabolism	31	31	31
RNA metabolism	56	56	56
Nucleosides and nucleotides	104	101	101
Protein metabolism	216	174	174
Cell division and cell cycle	4	4	4
Motility and chemotaxis	7	46	45
Regulation and cell signaling	30	28	28
Secondary metabolism	8	6	6
DNA metabolism	64	64	64
Fatty acids, lipids, and isoprenoids	47	48	49
Nitrogen metabolism	19	19	19
Dormancy and sporulation	96	97	97
Respiration	39	38	38
Stress response	45	47	47
Metabolism of aromatic compounds	13	12	12
Amino acids and derivatives	295	302	300
Sulfur metabolism	8	8	8
Phosphorus metabolism	11	11	11
Carbohydrates	245	243	243

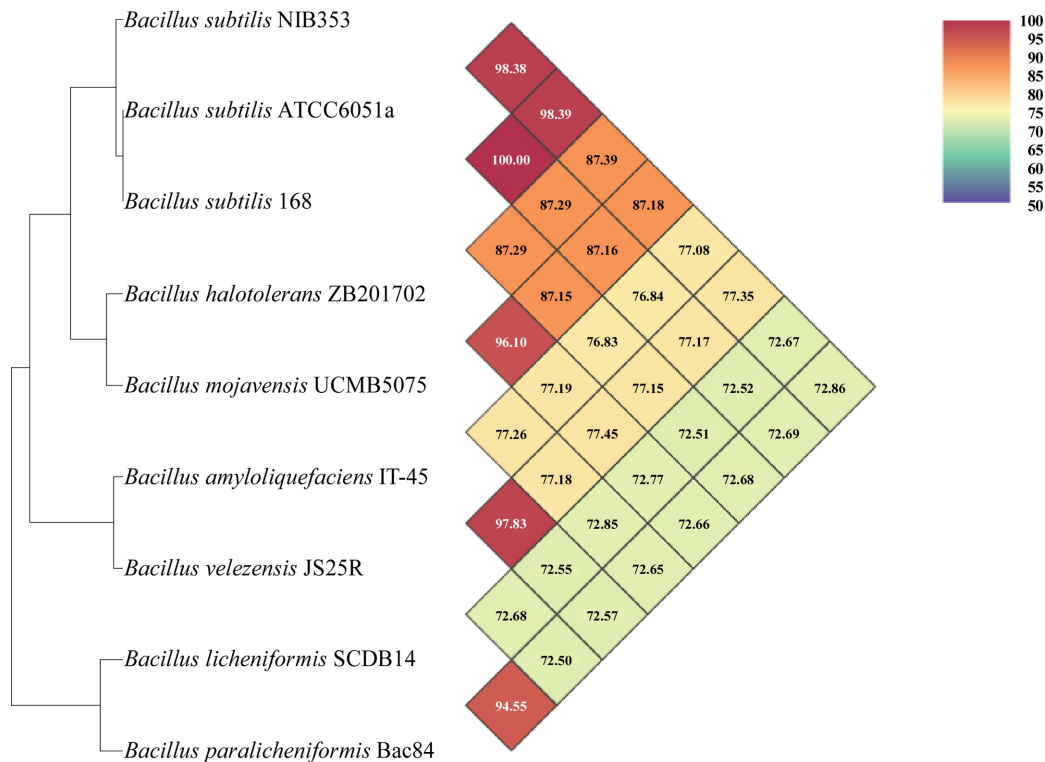


Fig. 1. Heatmap of the orthologous average nucleotide identity values of *Bacillus subtilis* NIB353 and other *Bacillus* species.

ANI values of 98.39% and 98.38% with *B. subtilis* 168 and *B. subtilis* ATCC6051a, respectively (Fig. 1). These insights into the genomic characteristics of *B. subtilis* NIB353 highlight its potential industrial use as a fermentation starter culture.

Nucleotide Sequence Accession Number

The complete *B. subtilis* NIB353 genome sequence has been deposited in the DDBJ/ENA/GenBank database under accession number NZ_CP089148.1.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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