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Complete Genome Sequence of *Bacillus safensis* DMB13 Exhibiting Non-Antibacterial Activity

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Strain *Bacillus safensis* DMB13 exhibiting protease and lipase activity was isolated from fermented kimchi in the previous study. Phenotypically, strain DMB13 showed no antibacterial activity. Thus, the complete genome sequence was analyzed to understand the phenotype of strain DMB13. The genome is 3,856,276-bp with a G+C content of 41.61 mol% and consists of a single circular 3,849,633-bp chromosome and one circular plasmids. Two genes related to bacteriocin production, *skfF* and *skfG*, were identified; however, six other genes in the *skf* operon were not detected. The genome includes 54 protease- and 5 lipase-encoding genes.

Keywords: *Bacillus safensis*, kimchi, bacteriocin, *skf* operon, protease, lipase

Bacillus safensis was initially discovered in the spacecraft-assembly facility at the Jet Propulsion Laboratory [1]. Since then, it has been isolated from various sources, including food products, such as honey, bread, and syrup. In addition to its presence in extreme environments, *B. safensis* shows high antimicrobial activity and is under investigation owing to its potential as a biopreservative in aquaculture [2, 3]. In a previous study, we isolated *B. safensis* from kimchi [4]. Antibiotic susceptibility testing and functional assays revealed four starter candidates out of 65 *Bacillus* strains, one of which was *B. safensis* DMB13 (initially designated as strain GN5_10) [4]. In this study, we evaluated the antimicrobial activity of strain DMB13. However, *B. safensis* DMB13 showed no antibacterial activity against nine species of foodborne pathogens, *Bacillus cereus* KCCM 11341, *Enterococcus faecalis* KCTC 2011, *Listeria monocytogenes* ATCC 13932, *Staphylococcus aureus* ATCC 12692, *Alcaligenes xylosoxidans* KCCM 40240, *Escherichia coli* O157:H7 EDL 933, *Flavobacterium* sp. KCCM 11374, *Salmonella enterica* KCCM 11862, and *Vibrio parahaemolyticus* KCTC 2729, contradicting previous findings indicating that *B. safensis* shows strong antibacterial activity [2, 3]. Therefore, to uncover genetic factors influencing the antibacterial properties the strain and explain variation among studies, we analyzed the genome of *B. safensis* DMB13. Additionally, through genomic analyses, we evaluated its susceptibility to antibiotics, lack of hemolysis, and protease and lipase activity.

Whole-genome sequencing of *B. safensis* DMB13 was conducted at CJ Bioscience, Inc. (South Korea) using the PacBio Sequel 10K system. Sequencing yielded 50,427 reads with a coverage of 522.37×, from which two contigs were generated using the FLYE assembler (version 2.8.3) in SMRT Link (PacBio). Annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline (version 4.6). Annotated genes were classified into functional categories using the Clusters of Orthologous Groups (COG) database [5], and metabolic pathways were examined using Rapid Annotation using Subsystem Technology [6].

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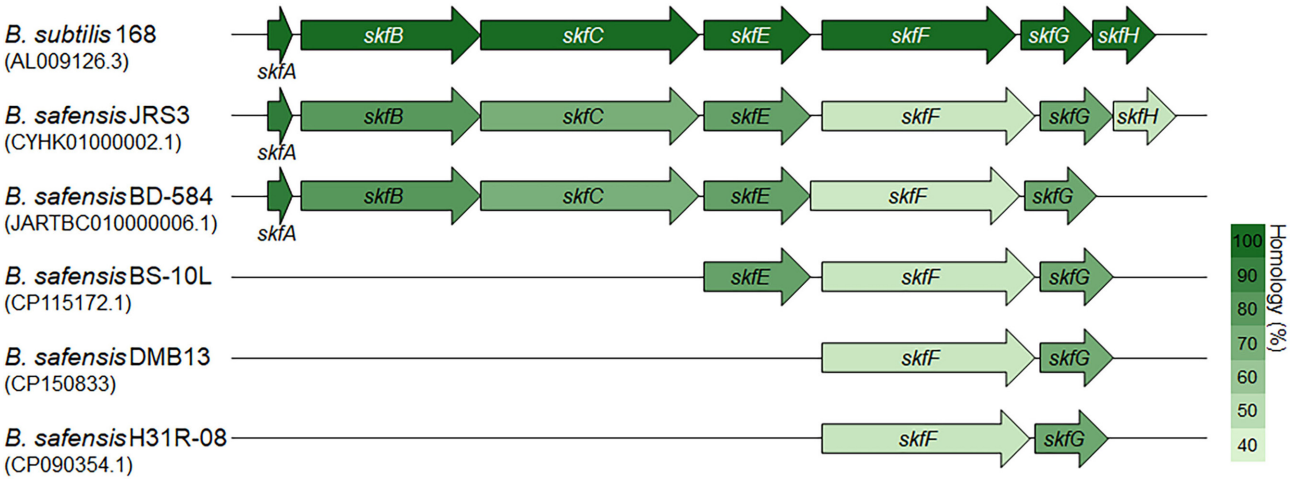

Fig. 1. Comparison of the *skf* (sporulation killing factor) operon in *Bacillus safensis* and *Bacillus subtilis*. The homology of each gene is color-coded based on comparisons with *B. subtilis* 168.

The complete genome of *B. safensis* DMB13 consisted of a circular 3,849,633 bp chromosome and a 6,643 bp plasmid pDMB13. The G+C content was 41.61 mol%. The average nucleotide identity between the genome of *B. safensis* DMB13 and those of *B. safensis* subsp. *safensis* FO-36b^T and *B. safensis* subsp. *osmophilus* CECT 9344^T were 97.29% and 96.25%, respectively. The genome harbored 3,968 open reading frames, including 3,862 protein-coding genes, 81 tRNA genes, 24 rRNA genes, and 1 additional RNA gene. Using COG, functional annotations were obtained for 3,561 genes, with significant enrichment for amino acid transport and metabolism (304 genes, 8.5%), transcription (291 genes, 8.2%), and carbohydrate transport and metabolism (255 genes, 7.2%). In a SEED subsystem analysis, 1,577 genes were annotated, and the most abundant subsystem category was amino acids and derivatives (280 genes, 17.8%), followed by carbohydrates (236 genes, 15.0%).

Although *B. safensis* strain DMB13 lacked antimicrobial activity, it contained genes related to the bacteriocin gene cluster, *skfF* and *skfG* (Fig. 1). However, the genome of strain DMB13 did not possess the entire operon necessary for the production of sporulation killing factor (*skf*) (Fig. 1). In contrast, *Bacillus subtilis* 168, reported to exhibit antimicrobial activity, had the *skf* operon (Fig. 1) [7]. Additionally, *B. safensis* JRS3 harbored the *skf* operon. The exact influence of Skf on interspecific antimicrobial activity is not well understood, although it is known to lyse sibling cells [8]. Nevertheless, if the sporulation killing factor generated from the *skf* operon is responsible for antimicrobial activity, the lack of antimicrobial activity in strain DMB13 can be attributed to the lack of the full-length *skf* operon.

B. safensis strain DMB13 exhibited sensitivity to eight antibiotics: ampicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, streptomycin, tetracycline, and vancomycin [4]. Specific antibiotic resistance genes for these eight antibiotics were not detected in the genome. Although hemolysis was not observed, a hemolysin-3 like protein gene (WNC56_10320) was detected. However, our previous research indicated that this gene is ineffective [9]. Consistent with these previous findings, hemolysis was not observed in this study, supporting the ineffectiveness of this gene.

B. safensis strain DMB13 exhibited lipase and protease activities and its genome possessed five lipase genes and 54 protease genes (Table 1). These genes may influence the lipase and protease activities of strain DMB13. This study provides a basis for further studies aimed at the identification of genes associated with the antimicrobial activity of *B. safensis* and provides insight into its functionality.

Nucleotide Sequence Accession Number

The complete genome sequence of *Bacillus safensis* DMB13 has been deposited in DDBJ/ENA/GenBank under accession number CP150833 and CP150834.

Table 1. Putative lipase and protease genes in the genome of *Bacillus safensis* DMB13.

Gene locus	E.C. no.	Product	COG	Gene
Lipase				
WNC56_02285	-	Spore germination lipase LipC	E	-
WNC56_02730	3.1.1.-	Hormone-sensitive lipase	I	*aes*
WNC56_13830	3.1.1.3	Triacylglycerol lipase	S	*lip*
WNC56_14175	3.1.1.5	Lysophospholipase	I	*pldB*
WNC56_16350	3.1.1.1	Carboxylesterase	S	*yvaK*
Protease				
WNC56_00285	-	Sporulation-specific protease YabG	S	-
WNC56_01390	3.4.21.-	Cell wall-associated protease	O	*wprA*
WNC56_01450	3.4.21.-	Minor extracellular protease Epr	O	*epr*
WNC56_01635	-	Cysteine protease StiP	S	-
WNC56_02530	-	Putative rhomboid protease YdcA	S	-
WNC56_02980	-	Putative membrane peptidase YdiL	S	-
WNC56_04470	3.4.21.89	Signal peptidase I	U	*lepB*
WNC56_04755	3.4.-.-	Probable peptidoglycan endopeptidase LytE	M|M	*lytE*
WNC56_05120	3.4.24.84	Ste24 endopeptidase	O	-
WNC56_05515	3.4.21.89	Signal peptidase I	U	*lepB*
WNC56_05975	3.4.24.-	Oligoendopeptidase F like protein	E	*pepF*
WNC56_06500	3.4.21.107	Peptidase Do	O	*degP*
WNC56_06545	3.4.14.13	Gamma-D-glutamyl-L-lysine dipeptidyl-peptidase	M	*ykfC*
WNC56_06660	3.4.21.-	Major intracellular serine protease	O	*isp*
WNC56_06755	3.4.24.-	Protease HtpX like protein	O	*htpX*
WNC56_06860	-	ATP-dependent Clp protease ATP-binding subunit ClpE	O	-
WNC56_07095	-	Putative L,D-transpeptidase YkuD	S	-
WNC56_07270	3.4.21.89	Signal peptidase I	U	*lepB*
WNC56_07720	3.4.21.-	Bacillopeptidase	O|S	*bpr*
WNC56_07725	3.4.23.-	Sporulation sigma-E factor-processing peptidase	S	*spoIIGA*
WNC56_07820	3.4.23.36	Signal peptidase II	MU	*lspA*
WNC56_08170	3.4.25.2	HslU--HslV peptidase	O	*hslV*
WNC56_08175	-	ATP-dependent protease ATPase subunit ClpY	O	-
WNC56_08380	3.4.24.-	Probable protease eep	M	*rseP*
WNC56_08535	3.4.24.-	Uncharacterized zinc protease YmxG	O	*pqqL*
WNC56_08580	3.4.21.92	Endopeptidase Clp	O	*clpP*
WNC56_08750	3.4.21.-	Serine protease AprX	O	*aprX*
WNC56_09020	3.4.21.19	Glutamyl endopeptidase	O	*sspA*
WNC56_10085	3.4.-.-	D-gamma-glutamyl-meso-diaminopimelic acid endopeptidase CwlS	M|M	*cwlS*
WNC56_10155	3.4.21.102	C-terminal processing peptidase	M	*prc*
WNC56_10900	-	Protease PrsW	S	-
WNC56_11525	3.4.21.116	SpoIVB peptidase	M	*spoIVB*
WNC56_11730	3.4.21.89	Signal peptidase I	U	*sipW*
WNC56_11825	3.4.19.11	Gamma-D-glutamyl-meso-diaminopimelate peptidase	E	*yqgT*
WNC56_11845	3.4.21.105	Rhomboid protease	S	*gluP*
WNC56_12190	3.4.24.78	GPR endopeptidase	O	*gpr*

Table 1. Putative lipase and protease genes in the genome of *Bacillus safensis* DMB13.

Gene locus	E.C. no.	Product	COG	Gene
WNC56_12595	3.4.-.-	Uncharacterized protease YrrO	O	-
WNC56_12600	3.4.-.-	Uncharacterized protease YrrN	O	-
WNC56_13060	3.4.21.53	Endopeptidase La	O	*lon*
WNC56_13065	3.4.21.53	Endopeptidase La	O	*lonB*
WNC56_13070	-	ATP-dependent Clp protease ATP-binding subunit ClpX	O	-
WNC56_13685	3.4.21.-	Putative signal peptide peptidase SppA	OU	*sppA*
WNC56_14205	3.4.14.5	Dipeptidyl-peptidase IV	E	-
WNC56_15220	-	Putative membrane protease YugP	S	-
WNC56_15640	-	Uncharacterized peptidase	E	-
WNC56_15700	3.4.-.-	L-Ala--D-Glu endopeptidase	M	*lytH*
WNC56_15950	3.4.21.107	Peptidase Do	O	*degP*
WNC56_16675	3.4.21.92	Endopeptidase Clp	O	*clpP*
WNC56_16745	3.4.-.-	Peptidoglycan DL-endopeptidase CwlO	M|S	*cwlO*
WNC56_16965	3.4.21.102	C-terminal processing peptidase	M	*prc*
WNC56_16975	3.4.-.-	Peptidoglycan DL-endopeptidase CwlO	S|M	*cwlO*
WNC56_18375	3.4.21.-	Minor extracellular protease vpr	O	*vpr*
WNC56_19440	3.4.21.107	Peptidase Do	O	*degP*
WNC56_19520	3.4.21.26	Prolyl oligopeptidase	S	-

The Enzyme Commission (EC) number is a numerical classification scheme for enzymes, based on the chemical reactions they catalyze. The EC numbers are based on the genes of strain DMB13 and gene are assigned by BlastKoALA. The Clusters of Orthologous Group (COG) categorization was generated by annotated gene functions.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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