

Complete genome sequence of *Bacillus halotolerans* F41-3 isolated from wild flower in Korea

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야생화로부터 분리한 *Bacillus halotolerans* F41-3 균주의 전체 게놈서열

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(Received September 9, 2019; Revised September 16, 2019; Accepted September 16, 2019)

A number of *Bacillus* strains are known to have antimicrobial activity useful in various fields. In order to prevent *Propionibacterium acnes*, which is one of the factors of acne, we selected *Bacillus halotolerans* F41-3 which have high antimicrobial activities against *P. acnes*. We conducted complete genome sequencing of *B. halotolerans* F41-3 and analyzed genomic characteristics. This genome size is 4,144,458 bp with a G + C content of 43.76%, 4,145 total genes and 3,686 protein coding genes. Among the genes, we found that gene cluster of subtilisin, a kind of bacteriocin, synthesis and gene cluster of nickel transportation. Both of them may influence inhibition of *P. acnes*.

Keywords: *Bacillus halotolerans*, genome sequence, inhibition of *Propionibacterium acnes*, subtilisin

Antibacterial activity is important especially for preventing pathogens. Some microbes and their antimicrobial substances were used for acne treatment as a natural antibiotic against *Propionibacterium acnes* which is a Gram-positive bacterium and one of the causative agents of acne (Lee and Song, 2018). A number of *Bacillus* strains were reported as antimicrobial bacteria against Gram-positive bacteria (Sumi *et al.*, 2015). In this study, several *Bacillus* strains were isolated from a variety

of flowers. The flower samples were suspended in distilled water and then spread and cultivated on R2A agar at 28°C for two days. Among them, *B. halotolerans* F41-3 which was isolated from a flower of Chinese redbud (*Cercis chinensis*) had higher antibacterial activity against *P. acnes* than other *Bacillus* strains. To investigate antibacterial factors in respect of genomics, we conducted whole genome sequencing of the *B. halotolerans* strain F41-3.

The genomic DNA was extracted using a Wizard genomic DNA isolation kit (Promega). Single Molecule, Real-Time (SMRT) library was prepared to sequence long fragments and sequencing was performed by *de novo* sequencing using PacBio RS II (Pacific Biosciences) at the Macrogen. *De novo* assembly was performed by using RS HGAP assembly version 3.0 (Chin *et al.*, 2013). The genome was annotated by NCBI prokaryotic genome annotation pipeline (Tatusova *et al.*, 2016), and submitted to the GenBank (<http://www.ncbi.nlm.nih.gov/>) database (CP041357).

The complete genome sequence of strain F41-3 was composed of a single circular chromosome of which genome coverage was 198.0 x and did not contain plasmid. The chromosome genome was 4,144,458 bp with a G + C content of 43.76% and contained 4,145 coding genes (CDS), 459 pseudo genes (404 genes are frameshifted), and 121 RNA genes. The genomic

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Table 1. Genome feature of *Bacillus halotolerans* F41-3

Attribute	Value
Genome size (bp)	4,144,458
GC content (%)	43.96
No. of contigs	1 (CP041357)
Total genes (CDS)	4,145
Protein-coding genes	3,686
tRNAs	86
rRNAs	30
ncRNAs	5
Pseudogenes	459
Plasmid	0

feature of strain F41-3 was summarized in Table 1.

We compared the ANI value between strain F41-3, and *B. halotolerans* ATCC 25096^T (LPVF01000000) and *B. mojavensis* KCTC 3706^T (AYTL01000000) which were the most closely related species on the basis of high 16S rRNA similarities (99.93% and 99.86%, respectively). The OrthoANI value was calculated using EZbiocloud (<http://www.ezbiocloud.net/tools/ani>) (Yoon *et al.*, 2017). Strain F41-3 was the highest OrthoANI value of 98.03% with *B. halotolerans* ATCC 25096^T and a value of 96.00% with *B. mojavensis* KCTC 3706^T, which meant the strain should be classified into *B. halotolerans* (Chun *et al.*, 2018).

Characteristically, strain F41-3 contained two gene clusters which were absent in the genome of type strain ATCC 25096^T; subtilisin (FLQ13_05815) and associated genes (FLQ13_05820 to FLQ13_05855), and nickel transporter system genes (FLQ13_00230 to FLQ13_00255). Subtilisin which was first reported to be produced by *Bacillus subtilis* strain 168 inhibited the growth of some Gram-positive bacteria (Babasaki *et al.*, 1985). The nickel transport system present in diverse microorganisms was reported that nickel into cell influenced formation of subtilisin (Kawulka *et al.*, 2004). Therefore, we assume that this may be the cause of high antibacterial activity.

Availability of the sequence data and strain

The complete genome sequence of *Bacillus halotolerans* F41-3 has been deposited to GenBank under accession number CP041357. The strain is available at the Korean Agricultural Culture Collection (accession number KACC 21447).

적 요

많은 *Bacillus* 속 균주들이 다양한 분야에서 이용할 수 있는 항균 활성을 가지고 있는 것으로 알려져 있다. 여드름을 유발하는 인자 중 하나인 *Propionibacterium acne*를 억제하기 위해 *P. acnes*에 대해 높은 항균활성을 가지는 *Bacillus halotolerans* F41-3 균주를 선별하였다. 우리는 *B. halotolerans* F41-3 균주에 대한 유전체 분석을 하였고 유전적 특성을 분석하였다. 이 유전체는 4,144,458 bp의 크기로 G + C 함량은 43.76%, 유전자의 수는 4,145개, 단백질 암호화 유전자는 3,686개로 구성되어 있다. 유전체 가운데 bacteriocin 중 하나인 subtilisin에 연관된 유전자 클러스터와 subtilisin 합성에 관여하는 nickel 수송에 연관된 유전자 클러스터가 포함되어 있는 것을 확인하였다. 두 클러스터가 *P. acnes*를 저해하는데 영향을 줄 것으로 보인다.

Acknowledgements

This study was carried out with the support (PJ013549) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

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