

# Complete genome sequence of *Bacillus licheniformis* CP6 strain possessing a proteolytic enzyme activity for the degradation of the plant-based raw materials


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## 식물 기반 소재의 분해를 위한 단백질 가수분해 능력을 가진 *Bacillus licheniformis* CP6 균주의 전장유전체 분석

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A *Bacillus licheniformis* CP6 strain was isolated from Tong-Yeong seawater in Korea for finding halo-tolerant bacteria possessing proteolytic enzyme activity. This strain can grow a diverse concentration of NaCl together with the production of protease. The complete genome comprises 4,316,909 bp with a G + C content of 46.0%, 4,276 coding genes, and 110 RNA genes, respectively. Strain CP6 was identified as *B. licheniformis* using the EZBioCloud offered in ChunLab and the RAST server analysis revealed it harbored 23 protein degradation-related genes, showing that it can be used as a source of the fermentation strain for the development of functional cosmetic ingredient by using plant-based raw materials.

**Keywords:** *Bacillus licheniformis*, fermentation, genome sequence, plant-based raw materials, proteolytic activity

Recently, microbial fermentation has been focused on the development of functional ingredients from natural raw materials especially plant-based raw materials. It can be also used to set up eco-friendly processes for the mass production of

ingredients in the cosmetic industrial field (Majchrzak *et al.*, 2022). It is used to enhance the biological activity of the end-product by converting high-molecular compounds into low-molecular structures and to produce *Bacillus* ferments as one of the cosmetic ingredients by cultivation with *Bacillus* sp. (Duarte *et al.*, 2022). Therefore screening and identification of bacteria possessing hydrolytic enzyme activity and selecting unique plant-based raw materials are needed to develop innovative ingredients in the cosmetic industry (Cruz-Casas *et al.*, 2021).

During the study of screening and phenotypic analysis for the isolation of bacteria with the production of protease, a bacterial strain CP6 showing a strong proteolytic enzyme activity was isolated. Based on phylogenetic analysis of 16S rDNA, strain CP6 was identified as a member of genus *Bacillus*. Here we describe the complete genome sequence and annotation of *Bacillus* sp. CP6 is used to degrade the powder of *Aurea helianthus*. It is one of the curative herbs and also known as wild confederate rose or golden sunflower has been used as a medicinal material in China due to its anti-inflammatory,

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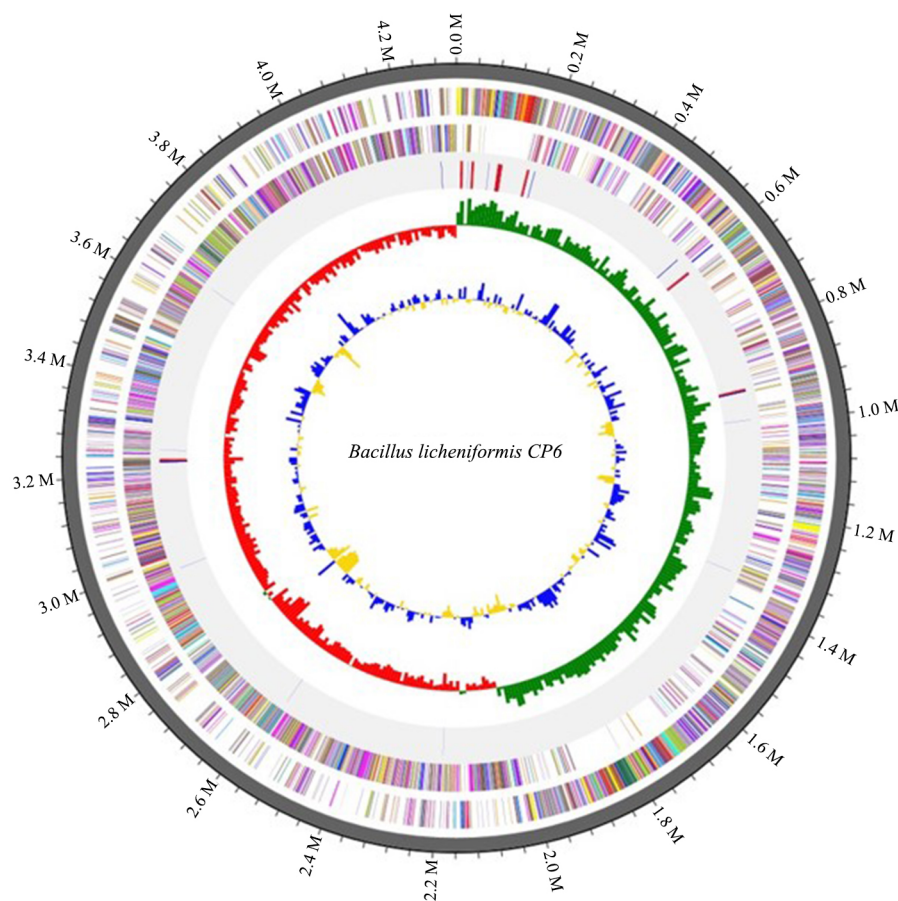
**Table 1.** General features of *Bacillus licheniformis* CP6

Property	Value
Genome assembly	
Assemble method	SMRT 2.3.0 (HGAP2)
Genome coverage	151.9×
Genome features	
Genome size (bp)	4,316,909
G+C content (%)	46.0
No. of contigs	1
Total genes	4,495
Protein-coding genes	4,276
Pseudo genes	109
rRNA genes (5S, 16S, 23S)	24 (8, 8, 8)
tRNA genes	81
GenBank Accession No.	CP049330

immune regulatory, and anti-oxidant activities (Kim *et al.*, 2018).

A single colony of CP6 strain was grown in the marine agar

medium (BD) which contained 3% sodium chloride for 3 days at 25°C under aerobic conditions. The genomic DNA was extracted using a standard Genomic DNA purification kit (Promega), and its quantity and quality were assessed by Agilent 2100 Bioanalyzer. The genome sequencing was performed using a single-molecule real-time (SMRT) sequencing platform on the PacBio RS II (Pacific Biosciences). The whole-genome sequence of strain CP6 was performed using a single SMRT cell (Pacific Biosciences) with P6C4 chemistry. The generated sequencing reads were de novo assembled using the hierarchical genome assembly process (HGAP) protocol RS HGAP Assembly 2 in SMRT analysis version 2.3.0. The coding sequences (CDSs) were predicted by Prokaryotic Genome Annotation Pipeline (PGAP) software on NCBI. The complete genome features of CP6 are summarized in Table 1. The genome size was 4,316,909 bp with 151.9× coverage and the GC content was 46.0%. Out of 4,495 predicted genes, 4,276



**Fig. 1.** Genome map of *Bacillus licheniformis* CP6. From the outermost circle to the inner, each circle contains information about (1) Contig, (2) Forward CDS, (3) Reverse CDS, (4) rRNA and tRNA, (5) GC Skew, and (6) GC Ratio.

were assigned as protein-coding genes (Table 1, Fig. 1). The 16S-based ID shows 99.66% of similarity with *Bacillus licheniformis* ATCC 14580<sup>T</sup> using EzBioCloud service offered in ChunLab (Yoon et al., 2017). Therefore strain CP6 was identified as *B. licheniformis* and *B. licheniformis* ATCC 14580<sup>T</sup> was the closest relative strain of CP6. Furthermore, the genome was annotated using Rapid Annotations using Subsystem Technology (RAST) server (Aziz et al., 2008). The RAST server predicted 334 classified subsystems with 27% of coverage. Amino acids and derivatives-related features (325 genes), carbohydrates (330 genes), protein metabolism (202 genes), cofactors, vitamins, prosthetic groups, pigments subsystem (148 genes), and dormancy and sporulation (100 genes) were the predominant subsystems in the strain. Among the subsystem of protein metabolism, 23 genes are involved in protein degradation, indicating that aminopeptidase (2 genes), protein degradation (2 genes), metalloprotease (8 genes), serine endopeptidase (1 gene), proteolysis in bacteria, ATP-dependent (7 genes), and omega peptidase (3 genes). These results suggest that the complete genome information of *B. licheniformis* CP6 will contribute to the understanding of the biological functions of *B. licheniformis* CP6 strain for the develop innovative ingredients together with plant-based raw materials in the cosmetic industry for the eco-friendly industrial application.

#### Nucleotide sequence accession number

*Bacillus licheniformis* CP6 has been deposited in the Korean Collection for Type Cultures under accession number KCTC 18811P. The GenBank/EMBL/DDBJ accession number for the genome sequence of *B. licheniformis* CP6 is CP049330.1.

## 적 요

본 연구는 국내 통영 바닷물에서 다양한 NaCl 농도에서 생육이 가능하며 단백질 분해 효소 활성이 높은 내염성의 미생물 *Bacillus licheniformis* CP6 균주를 분리하고 유전체서열을 PacBio RS II 플랫폼을 사용하여 분석하였다. 염색체의 크기는 4,316,909 bp로 G + C 구성 비율은 46.0%, 코딩 유전자수는 4,276개, RNA 관련 유전자는 110개로 구성되어있다. CP6 균

주는 천랩에서 제공하는 EZBioCloud service를 통하여 *B. licheniformis*로 확인되었으며 RAST server을 활용한 유전체 분석을 통하여 23개의 protein-degradation 관련 유전자가 존재하는 것을 확인하였기에 *B. licheniformis* CP6 균주는 기능성 화장품 소재 개발을 위한 식물 기반 소재 물질의 발효 균주로 활용될 수 있을 것이다.

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## Conflict of Interest

The authors have no conflict of interest to report.

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