# Antimicrobial Activity of Bacillus sp. K-9 Against Potato Scab and Its Genome-wide Analysis

**Ma Shuang** ( ndms1022@163.com )
Heilongjiang Bayi Agricultural University
**Wang Teng**
Heilongjiang Bayi Agricultural University
**Jiarong Ru**
Heilongjiang Academy of Agricultural Sciences
**Lili Jiang**
Heilongjiang Bayi Agricultural University
**Yanjie Wang**
Heilongjiang Bayi Agricultural University
**Cong Wang**
Heilongjiang Academy of Agricultural Sciences

**Research Article**

**Keywords:** Bacillus sp. K-9, Potato scab, Complete genome sequence, Gene function

**Posted Date:** April 15th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1549183/v1

## Abstract

We isolated a bacterial strain K-9, identified as a novel species of the genus *Bacillus*, from a potato tuber sample. The inhibition rate of the strain K-9 against potato scab (*Streptomyces scabies*) was 44.90%. The whole genome sequence of *Bacillus* sp. K-9 was obtained, and genomic analyses were performed. Complete genome sequence of strain K-9 was obtained through PacBio RSII and Illumina platforms. The size of genome is 3891530 bp, with estimated 3915 coding DNA sequences, GC content of 46.45%, 79 tRNA, and 10 rRNA. In addition, functional annotation of the strain K-9 genes was performed by GO, COG, KEGG, and CAZy analyses. There were 12 gene clusters for secondary metabolite synthesis in the genome of *Bacillus sp.* K-9. Except for the unknown metabolites of the T3PKS (third polyketone), terpene and lanthipeptide class-II, the other eight gene clusters are associated with the synthesis of secondary metabolites that are common in *Bacillus* and have antibacterial activity. Among them, the homologous alignment function types are mostly related to the synthesis of antibacterial active substances such as PKS (polyketone synthase), NRPS (non-ribosomal peptide synthase) and bacilysin. In the future, information derived from the thorough genomic analyses of this strain may be helpful in directed genetic modification of bacterial strains for production of antibacterial substances to control potato scab.


## Introduction

Potato (*Solanum tuberosum L.*) is rich in carbohydrates and a variety of minerals and nutrients needed by humans (Majeed et al. 2017). Potato plays an important role in economy and global food security. However, with the rapid development of potato industry, the incidence and severity of scab have been increasing year by year due to the continuous planting of potatoes, which negatively influenced the development of potato industry. Potato scab, as a disease spread by soil and seed potato, is widespread in the potato-growing areas (Lankau et al. 2020). Potato scab is widely distributed in the world, and has been found in Argentina and other South American countries, the United States, Germany, Mexico, Finland, Japan, and South Korea, as well as in Yunnan, Heilongjiang and Shandong provinces of China (Joshi et al. 2010; Cheng et al. 2019; Hiltunen et al. 2021; Chater 2016; ). Potato scab can invade plants through skin cuts, wounds or young tubers, and can infect also other root crops such as radish, beet, carrot, and parsnip (Flores et al. 2010; Santos-Cervantes et al. 2017).

At present, chemical pesticides are difficult to be replaced, but the improper use of chemical drugs not only produces harmful effects on plants, but also leads to human and animal poisoning, killing beneficial microorganisms, leading to drug resistance of pathogens. And in many cases chemical drugs cannot completely cure plant diseases. The use of antagonistic bacteria to reduce or replace the use of pesticides and fertilizers is of great significance for maintaining the biodiversity of plant microecosystems, maintaining ecological balance and realizing sustainable development.

## Materials And Methods

### Pathogen and antagonistic bacteria

*Streptomyces scabies* (*S. scabies*) was isolated and preserved by plant pathology laboratory of Heilongjiang Bayi Agricultural University. *S. scabies* cultured in yeast malt agar medium (YME) (yeast extract 4 g, malt extract 10 g, glucose 4 g, sterile water 1000 mL, pH 7.2). The cultures were stored at -80℃.

Strain *Bacillus sp.* K-9 was isolated from potato tuber. Tuber homogenate dilution was spread on beef extract medium (NA) plates (beef extract 3 g, peptone 5 g, yeast extract 1 g, sucrose 10 g, agar 17 g, sterile water 1000 mL, pH 7.2). The isolates were purified and cultured at 28℃and stored at -80℃.

### Antimicrobial activity of *Bacillus sp.* K-9 against potato scab

Antagonistic strains were screened by the dual culture method (Cui et al. 2019). *Streptomyces scabies* was cultured on YME plates for 7 d and then inoculated in YME liquid medium at 30 °C, 180 r/min for 72 h to prepare the *S. scabies* suspension. The bacterial suspension was filtered through the filter membrane (0.22 μm) into the sterilizing tube to obtain sterile fermentation liquid for use. Then, *Bacillus sp.* K-9 strain, bacterial suspension and sterile filtrate were antagonized by plate. The antagonistic bacteria were then inoculated on the YME plates (85 mm) containing 200 μL of *S. scabies* suspension. The plates containing only *Streptomyces scabies* were used as the control. The inhibition rate of bacterial strains was determined. The antibacterial activity of antagonistic bacteria was evaluated by measuring the diameter of inhibition zone. Bacteria inhibitive rate (%) = bacteriostatic diameter/plate diameter×100.

### Extraction of genomic DNA from *Bacillus sp.* K-9

*Bacillus sp.* K-9 single colony was cultured in LB liquid medium on a shaking table at 28 ℃ for 12 h. The antagonistic bacterium was further identified through the analysis of its 16S rDNA gene sequences (Fuzhen, 2020; Rushabh, 2020; Tuo, 2020; Wei, 2020). DNA of antagonistic strains was extracted by a Tiangen Bacterial genome kit. The 16S rDNA primers were 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR amplification of 16S rDNA was done in a mixture containing PCR MIX 25 μL, Forward and reverse primers 1 μL each, and bacterial DNA 2 μL. The PCR reaction procedure was: pre-denaturation at 95 °C for 5 min; 95 °C denaturation for 30 s, 57 °C annealing for 30 s, 72 °C extension for 45 s, 30 cycles; the final elongation was at 72 °C for 7 min. The products were sequenced by Sangon Biotech Co. Ltd (Shanghai, China). The sequencing platform of *Bacillus sp.* K-9 genome (including plasmid) was PacBio RS II. After BLAST comparison on NCBI, strains with high similarity in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were selected, and the neighbor-joining method in MEGA 7 software was used to construct a phylogenetic tree. The nucleotide sequences were deposited in GenBank database. The GenBank accession number of the 16S rDNA gene sequence of strain K-9 is OL378201. The GenBank accession number of complete genome sequence of strain K-9 is JAKQYO000000000.

### Genomic DNA sequencing and assembly

An Illumina sequencer was used to sequence DNA. The HiSeq raw sequences containing adapter/primers were filtered out using FastQC (V0.11.9). Trimmomatic was used to perform quality shearing on Illumina sequencing data. SPAdes was used to splice second-generation sequencing data, and Gap Filler was used to fill gaps in contigs. PrInSeS-Gis was used for sequence correction to correct editing errors and insertion loss of small fragments in the splicing process.

**Gene prediction and annotation**

After extracting the coding genes, Interprosca was used to extract the annotation information of GO database. Blastp was used to compare the encoded proteins to KEGG database, and the best result with a comparison coverage greater than 30% was retained as the annotation result. The encoded proteins were annotated to the COG database using rpsblast. The gene protein sequences were compared with CAZy database by HMMER3, and the functional annotation information was obtained. Using the online prediction software antiSMASH6.0.1 (https://antismash.secondarymetabolites.org/#! /start) the secondary metabolite synthesis gene cluster of *Bacillus sp.* K-9 was analyzed to find the potential inhibitory metabolite synthesis genes.

**Strain K-9 produced ferriophagic activity, lipopeptide crude and protein extracts antibacterial activity**

Determination of iron-phagogic Activity in Strain K-9 by CAS(Chrome Azurol S) Agar Plate Assay (Ames,1989). The crude extract was obtained by acid precipitation method, and 100 µL was used for identification of antagonistic activity.Crude protein extract was obtained by ammonium sulfate precipitation method, and 100 µL was used for antagonistic activity identification (Wang, 2020).

**Statistical analysis**

Statistical analysis was performed using SPSS software(IBM SPSS Statistics, Version 22.0, International Business Machines Corporation, New York, in USA). Mean values were compared using Duncan's new multiple range test at the 1% ($P< 0.01$) level of significance between treatments.

## Results And Discussion

Bacillus sp. **K-9 antagonizes** S. scabies

The antagonistic activity to *S.scabies* was screened by the double culture method. The maximum antibacterial rate of strain K-9 was detected for fermentation liquid, and the minimum antibacterial activity was in cell-free filtrate. The antibacterial rate of fermentation broth was 53.92%. The antibacterial rate of strain K-9 was 44.90% and of cell-free filtrate was 27.25% (Fig. 1).

**Genomic identification of** Bacillus sp. **K-9**

The length of 16S rDNA of strain K-9 was 1447 bp. The 16S rDNA gene sequence of strain K-9 was related to *Bacillus velezensis*, *Bacillus amyloliquefaciens* and *Bacillus subtilis* and other strains, sharing more than 97% of the genetic sequences. The results of phylogenetic tree showed that K-9 and *Bacillus sp.* SA3 strain branched closely, with 63% similarity rate.

In the CGView circle of *Bacillus sp.* K-9 genome (Fig. 1), corresponding information from outer circle to inner circle is as follows: the first circle (outermost circle), GC content; circle 2, sequencing depth; circle 3, gene category; the fourth circle, CDS information on the positive chain; circle 5, CDS information on the negative chain; the sixth circle, negative chain COG functional classification annotation information; circle 7 (innermost circle), plus link COG functional classification annotation information.

The genome size was 3 891 530 bp. The GC content was 46.45%, and there were 11 contigs. The number of tRNA and rRNA was 79 and 10, respectively. There were 3915 protein-coding genes in *Bacillus sp.*K-9, and the proportion of protein -coding genes was 89.87%.

Table 1
Complete genome features of *Bacillus sp.*K-9

| Property | Value |
|---|---|
| Sequencing platform | PacBio RS II |
| Genome size (bp) | 3891530 |
| Contig numbers | 11 |
| DNA G + C (%) | 46.45 |
| Number of tRNA genes | 79 |
| Number of rRNA genes | 10 |
| Number of protein coding genes | 3915 |
| Ratio of protein coding genes | 89.87 |

Annotation of the Clusters of Orthologous Groups (COG) of proteins has been developed based on protein sequence alignment based on gene sequences, with each COG representing a gene family (Radisic et al. 2021; Paolo et al. 2020). The COG classification number of *Bacillus sp.* K-9 genome was 4, and the number of COG types was 20. The number of genes with COG annotation was 1238, accounting for 33.05% of all genes. As can be seen from COG annotation

information (Fig. 4), apart from the predicted gene function represented by "R" and the unknown function represented by "S", "K" represents the transcription gene family with the largest number of members (239), followed by "E" representing the amino acid transfer and metabolism family with 238 members.

Through genomic GO (Gene Ontology) annotations, biological terms can be standardized for easy communication. Statistical results of GO annotation of *Bacillus sp.* K-9 genome are shown in Fig. 5. In *Bacillus sp.* K-9 genome, the number of GO classifications was 3, and the number of genes involved in annotation was 3004, accounting for 80.19% of all genes.

In the KEGG (Kyoto Encyclopedia of Genes and Genomes) annotations of *Bacillus sp.* K-9 genome, the gene functions were divided into six major categories. Among them, 82 genes were annotated to cellular processes, 374 genes to environmental information processing, 240 to genetic information processing, 2066 to metabolism, 68 to human diseases, and 28 genes to organismal systems. Detailed breakdown of each category is shown in Fig. 6.

As shown in Fig. 7, among the genes coding for fermentation activity, the glycoside hydrolase genes were the most abundant in *Bacillus sp.* K-9, accounting for 29.65%, followed by 48 genes of glycosyl transferases, accounting for 27.90%. The gene numbers for carbohydrate esterases, carbohydrate-binding modules, auxiliary oxidoreductases, and polysaccharide lyases were 39 (22.67%), 18 (10.47%), 11 (6.40%), and 5 (2.91%), respectively. The main components of plant diseases and residues in soil are sugars and proteins, and the strain K-9 contains β-glucosidase (EC3.2.1.21), chitin (β-1,6-glucanosyltransferase, EC 2.4.1.-), α-amylase (EC 3.2.1.1), cellulase (EC 3.2.1.4) and other enzymes. The biocontrol mechanism of strain K-9 was further analyzed from the perspective of gene.

AntiSMASH6.0.1 software was used to predict the secondary metabolites of *Bacillus sp.* K-9. The results showed that there were 12 secondary metabolite synthesis gene clusters in the genome of *Bacillus sp.* K-9. Except for the unknown metabolites of the third polyketone (T3PKS), terpene and lanthipeptide-class-II, the other eight gene clusters are associated with biosynthesis of secondary metabolites that have antibacterial activity and are common in *Bacillus*: Clusters 1 and 5, polyketone synthesis genes; Cluster 4, synthesis gene cluster of Camelina; Cluster 6, macrolide synthesis; Cluster 9 butamectin synthesis; Cluster 10, lysobacterin synthesis; Cluster 11, siderophores synthesis(Fig. 8-A); and Cluster 12, surfactant synthesis. Gene clusters 4, 11 and 12 are associated with synthesis of secondary metabolites through non-ribosomal pathways (Table 2, Fig. 8-B).

Table 2
Prediction of secondary metabolites of *Bacillus sp.*K-9 strain

| Gene cluster[a] | Type[b] | Location[c] | Secondary metabolites[d] | Function[e] | Similarity[f] |
|---|---|---|---|---|---|
| 1 | TransAT-PKS | 546640–638999 | Difficidin | Anti-bacterial | 100% |
| 2 | T3PKS | 767634–808360 | — | — | — |
| 3 | Terpene | 873700–893826 | — | — | — |
| 4 | NRPS | 922563–1056873 | Fengycin | Anti-fungal | 100% |
| 5 | TransAT-PKS | 1130615–1231180 | Bacillaene | Anti-fungal, anti-bacterial | 100% |
| 6 | TransAT-PKS | 1450368–1538601 | Macrolactin | Anti-fungal, anti-bacterial, anti-virus | 100% |
| 7 | Lanthipeptide-class-ii | 1705164–1734052 | — | — | — |
| 8 | Terpene | 1854545–1875285 | — | — | — |
| 9 | PKS-like | 1957329–1998573 | Butirosin | Anti-bacterial | 7% |
| 10 | Other | 83195–124613 | Bacilysin | Anti-bacterial | 100% |
| 11 | NRPS | 660923–712714 | Bacillibactin | Accumulate and take up iron ions | 100% |
| 12 | NRPS | 199691–265098 | Surfactin | Anti-virus, anti-mycoplasma,anti-tumour | 82% |

a : the secondary metabolite synthesis gene cluster annotated with antiSMASH6.0.1, b : the gene cluster type,c : the location of gene clusters in the strain genome, d : the secondary metabolite that may be produced based on the gene cluster, e : the bioactive function of metabolites synthesized by gene cluster, f : similarity to known gene clusters,"—": indicates unknown.

Antibacterial peptide Lcl gene exists in strain K-9, and it has been reported (Chen,1996) that antibacterial peptide Lcl protein in Bacillus can effectively inhibit the growth of pathogens, and it is speculated that secretion of antibacterial substances in strain K-9 is one of its antibacterial mechanisms. The specific gene sequence of Lcl in K-9 genome is ATGAACTTCAAAAAAGTGTTAACCGGTTCTGCGCTGTCTC
TTGCCTTACTGATGTCTGCCGCTCCTGCCTTTGCCGCATCACCAACAGCATCCGCATCCATGGAAAATAGCCCAATCTCAACGAAAGCAGATGCCGGCATTAATGCCATCA

The specific protein sequence of LCl in k-9 is:MNFKKVLTGSALSLALLMS
AAPAFAASPTASASMENSPISTKADAGINAIKLVQSPNGNFAASFVLDGTKWIFKSKYYDSSKGYWVGIYESVDK. Antimicrobial peptide LCl protein sequences in K-9 were compared online in NCBI database. The results showed that the similarity of antimicrobial peptide LCl protein with *Bacillus* (WP_032872774.1) was 100%. It has been reported that LCl protein of *Bacillus subtilis* has significant effects on Xanthomonas campestris PV Oryzea, Pseudomonas Solanacearum PE1 is one of the main effective ingredients with strong antibacterial activity(Gong 2011). According to the bacteriostatic activity test results of crude protein extract of K-9 against Scab and protein gene analysis (Figure 8-C), it was speculated that the protein bacteriostatic activity substance of K-9 against Scab was LCl protein and the bacteriostatic gene was Lcl gene.

## Conclusion And Discussion

The problems of high residue, high pollution and harm to human and livestock caused by the use of chemical pesticides have become increasingly prominent.Green prevention and control technology has become a research hotspot, among which the application of biological antagonism to control various diseases has been proved to be a safe and effective new method.*Bacillus* has good control effect on crop diseases in biological control field. Traditional experimental and identification methods are difficult to fully analyze the antimicrobial substances of *Bacillus*, and can not fully explore all its antimicrobial genes. In order to further study this strain, the whole genome sequencing and bioinformatics analysis techniques were used to obtain the genome sequence of strain K-9. The genome size of strain K-9 was determined to be 3 870 130 bp, and a total of 4 161 genes were encoded. Meanwhile, the genome data were compared and analyzed with GO, COG, KEGG and other databases. The functional annotation and data statistics of K-9 genome were completed, and the classification status of K-9 was determined by 16S rDNA gene, and the gene clusters of secondary metabolites and antibacterial proteins that might play a role in its antibacterial action were explored from the perspective of molecular biology. The CAZy family of *Bacillus* sp. K-9 contains genes related to β-1, 3-glucanase, chitinase and other cell wall degradation enzymes. It is speculated that the cell wall degradation of pathogenic bacteria is one of the antibacterial mechanisms of strain K-9.

*Bacillus* is an excellent resource of biocontrol bacteria. Its secondary metabolites are rich, including antibacterial proteins, lipopeptides, polyketones and other antibacterial substances. The gene clusters of secondary metabolites of strain K-9 were predicted, and 12 gene clusters of lipopeptide and polyketone antibiotics were obtained. There were 4 unknown gene clusters, 7 antibiotic synthesis gene clusters (Difficidin,Fengycin, Bacillaene, Macrolactin,Bacilysin, Bacillibactin, and Surfactin) with high similarity, and 1 gene cluster with low similarity 7% antibiotic gene cluster (Butirosin). After homology comparison of functional types, *Bacillus sp.* K-9 contained multiple gene clusters related to antibacterial active substances such as PKS (polyketone synthase), terpene, NRPS (non-ribosomal peptide synthase), and bacilysin. These gene clusters may be involved in the anti-pathogen activity. Freitas et al. (2021) conducted a whole-genome analysis of *Bacillus* 64 − 1 and found that polyketo synthase (PKS), non-ribosomal peptide synthase (NRPS) and bacteriolysin may be related to antibacterial activity. Zeng et al. (2020) predicted the function of *Bacillus pumilus* GLB197 gene. Six gene clusters were related to the antibacterial activity of *Bacillus pumilus* GLB197, and NRPS gene clusters were predicted to be used in the production of antibiotics. Xu et al. (2020) sequenced the genome of *Bacillus amylolitica* HAB-2 and found that 13 gene clusters (NRPS and PKS, etc.) were involved in antibacterial action. In summary, the related gene clusters (NRPS, PKS and bacteriocin, etc.) that control the synthesis of active substances that are important to the anti-pathogenic activity of *Bacillus*.

The similarity of Butirosin antibiotic gene cluster found in this study was only 7%, indicating that this gene cluster may synthesize new antibacterial substances, which needs further study. It was predicted that strain K-9 could prevent scab by producing cell wall hydrolases, antagonistic active compounds and bacteriostatic proteins, which had great application potential in agriculture.

## Declarations

**Funding** This research was supported by The Integrated Research and Demonstration of Potato Fertilizer and Pesticide Reduction Technology of the National Researchand Development Program of China(2018YFD020080706).

### Author contributions

MaShuang wrote the main manuscript text and WangTeng give modification suggestions and data processing , Jiarong Ru prepared figures 2, Lili Jiang prepared table 1,Wang Cong prepared figures 1 and Yanjie Wang prepared language change . All authors reviewed the manuscript.

### Competing interests

The authors declare that they have no competing interests.

### Credit author statement

All of the authors have read and approved the manuscript. This work has not been published previously, nor is it being considered by any other peer-reviewed journal.

## References

1. Ames Gottfred N P, Christie B ï¼², Jordand C. (1989 ) Use of the Chrome Azurol S Agar plate technique to differentiate strains and field isolates of ï¼²hizobium leguminosarum biovar trifolii. Applied and environmental microbiology (5): 707–710. http:// med.wanfangdata. com. cn/Paper /Detail/PeriodicalPaper_PM16347877
2. Chater KF (2016). Recent advances in understanding *Streptomyces*. F1000 Research 5:2795. http://doi.org/10.12688/f1000research.9534.1
3. Cheng Z, Bown L, Piercey B, Bignell DR (2019) Positive and negative regulation of the virulence-associated coronafacoyl phytotoxin in the potato common scab pathogen *Streptomyces scabies*. Molecular Plant-Microbe Interactions32:331–356. http:// doi.org /10.1094/MPMI-03-19-0070-R
4. Cui LX, Yang CD, Wei LJ, Li TH, Chen X (2019) Isolation and identification of an endophytic bacteria bacillus velezensis 8 – 4 exhibiting biocontrol activity against potato scab. Biological Control 141:1–20. http://doi.org/10.1016/j.biocontrol.2019.104156
5. Cui Lingxiao,Yang Chengde,Wang Yinyu, et al.(2021) Potential of an endophytic bacteria *Bacillus amyloliquefaciens* 3–5 as biocontrol agent against potato scab. Microbial Pathogenesis(prepublish) 163:105382. https://doi.org/ 10.1016/J.MICPATH.2021.105382
6. Chen Ying, Li Ling, Han Yifan.(1996).Transformation of Antibacterial Gene Lcl into Poplar Species. Forest Research,(06):646–649. https://www.doc88.com/p-9425192677544.html?r=1

7. Flores GR, Velasco I, Montes(2010) Detection and characterization of *Streptomyces* causing potato common scab in western europe. Plant Pathology 57(1):162-169. https://doi.org/ 10.1111/j.1365-3059.2007.01734.x
8. Fuzhen Yang,Ruyang Zhang,Xianyu Wu, et al.(2020) An endophytic strain of the genus *Bacillus* isolated from the seeds of maize (Zea mays L.) has antagonistic activity against maize pathogenic strains. Microbial Pathogenesis 142:104074. https://doi.org/10.1016/ j.micpath. 2020.104074
9. FREITAS-SILVA J, DE OLIVEIRA B F R, VIGODER F M, et al.(2021) Peeling the Layers Away:The Genomic Characterization of *Bacillus pumilus* 64 – 1, an Isolate With Antimicrobial Activity From the Marine Sponge Plakina cyanorosea (Porifera, Homoscleromorpha)[J]. Frontiers in Microbiology 11: 592735. https://doi.org/10.3389/FMICB.2020.592735
10. Gong Weibin,Wang Jinfeng,Chen Zhangliang,Xia Bin,Lu Guangying(2011). Solution structure of LCI, a novel antimicrobial peptide from Bacillus subtilis. [J]. Biochemistry, 50(18): 3621–3627.http://dx.doi.org/10.1021/bi200123w
11. Hiltunen LH, Tarvainen O, Kelloniemi J, Tanskanen J, Karhu J, Valkonen JPT(2021) Soil bacterial community in potato tuberosphere following repeated applications of a common scab suppressive antagonist. Applied Soil Ecology 167:1-11.https://doi.org/10.1016/j.apsoil.2021. 104096
12. Joshi MV, Mann SG, Antelmann H, Widdick DA, Fyans JK, Chandra G, Hutchings MI, TothI, Hecker M, Loria R(2010) The twin arginine protein transport pathway exports multiple virulence proteins in the plant pathogen *Streptomyces scabies*. Molecular Microbiology 77: 252–271. https://doi.org/10.1111/j.1365-2958.2010.07206.x
13. Lankau EW, Xue D, Chrisensen R, Gevens AJ, Lankau RA(2020) Management and soil conditions influence common scab severity on potato tubers via indirect effects on soil microbial communities. Phytopathology 110:1-32. https://doi.org/10.1094/ PHYTO- 06-19 - 0223-R
14. Lingxiao Cui, Chengde Yang, Lijuan Wei, et al.(2020) Isolation and identification of an endophytic bacteria *Bacillus velezensis* 8-4 exhibiting biocontrol activity against potato scab. Biological Control 141:104156. https://doi.org/10.1016/j.biocontrol.2019.104156
15. Majeed A, Muhammad Z, Ullah Z, Ullah R, Ahmad H (2017) Late blight of potato(Phytophthora infestans) I: fungicides application and associated challenges. Food Science and Technology 5:261–266. https://doi.org/10.24925/turjaf.v5i3.261-266.1038
16. Paolo Stincone, Flávio Fonseca Veras, Jamile Queiroz Pereira, et al.(2020) Diversity of cyclic antimicrobial lipopeptides from *Bacillus* P34 revealed by functional annotation and comparative genome analysis. Microbiological Research(prepublish) 238:126515. https:// doi.org/10.1016/j.micres.2020.126515
17. Rushabh Shah, Natarajan Amaresan, Prittesh Patel, et al.(2020) Isolation and Characterization of *Bacillus sp.* Endowed with Multifarious Plant Growth-Promoting Traits and Their Potential Effect on Tomato (Lycopersicon esculentum) Seedlings. Arabian Journal for Science and Engineering 45:4579–4587. https://doi.org/10.1007/s13369-020-04543-1
18. Radisic Vera, Marathe Nachiket P.(2021) Genomic characterization of multidrug-resistant *Bacillus toyonensis* strain 4HC1 isolated from marine plastic in Norway. Journal of Global Antimicrobial Resistance(prepublish) 26:245–251. https://doi.org/10.1016/j.jgar.2021.07.002
19. Tuo Li, Liu Fang,Yan Xiao-Rui et al.(2020) *Bacillus taxi sp.* nov., a novel endophytic bacterium isolated from root of Taxus chinensis (Pilger) Rehd. International journal of systematic and evolutionary microbiology 70(1):481–486. https://doi.org/10.1099/ijsem.0.003776
20. Santos-Cervantes ME, Felix-Gastelum R, Herrera-Rodríguez G, Espinoza-Mancillas MG, Mora-Romero AG, Leyva-López NE.(2017) Characterization, pathogenicity and chemical control of *Streptomyces* acidiscabies associated to potato common scab.American Journal of Potato Research 94:14–25. https://doi.org/10.1007/s12230-016-9541-5
21. Weihui Xu, Kexin Wang, Hengxu Wang, et al.(2020) Evaluation of the biocontrol potential of *Bacillus sp.* WB against Fusarium oxysporum f. sp. niveum. Biological Control 147(C):104288. https://doi.org/10.1016/j.biocontrol.2020.104288
22. Wang Rui, Hu Huifan, Chen Wenlan, Zhang Qiang & Liu Baifang.(2020). Identification of antimicrobial activity of a strain of Bacillus methylotrophic. China Condiment (09):5–10. http://www.doc88.com/p-60999055098688.html
23. Xu P, Xie S, Liu W, et al.(2020) Comparative Genomics Analysis Provides New Strategies for
24. Bacteriostatic Ability of *Bacillus velezensis* HAB-2. Frontiers in Microbiology 11:594079. https://doi.org/10.3389/FMICB.2020.594079
25. Zhang Xiao-Yu, Li Chi, Hao Jian-Jun, et al.(2020) A Novel Streptomyces sp. Strain PBSH9 for Controlling Potato Common Scab Caused by *Streptomyces galilaeus*. Plant disease 104(7):1986–1993. https://doi.org/10.1094/PDIS-07-19-1469-RE
26. ZENG Q, XIE J, ZHANG X, et al.(2020) Complete genome sequence data of *Bacillus pumilus* GLB197, an effective antagonist of grape downy mildew. Data in brief 30: 105423. https://doi.org/10.1016/j.dib.2020.105423

## Figures

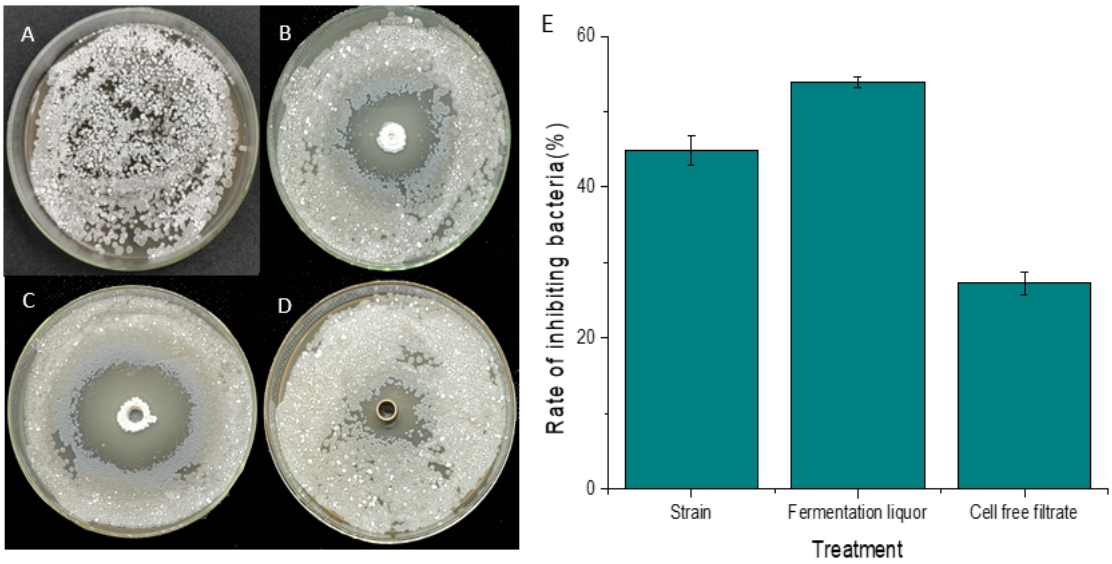


**Figure 1**

Antimicrobial activity of strain K-9, fermentation broth and cell free filtrate against *Streptomyces scabies*. A, B, C, D, E: Control of *S.scabies*, Dual-culture of strain K-9 against *S.scabies*, Strain K-9 fermentation liquid against *S.scabies*, Strain K-9 cell free filtrate against *S. scabies*, Bacteriostatic ratio.

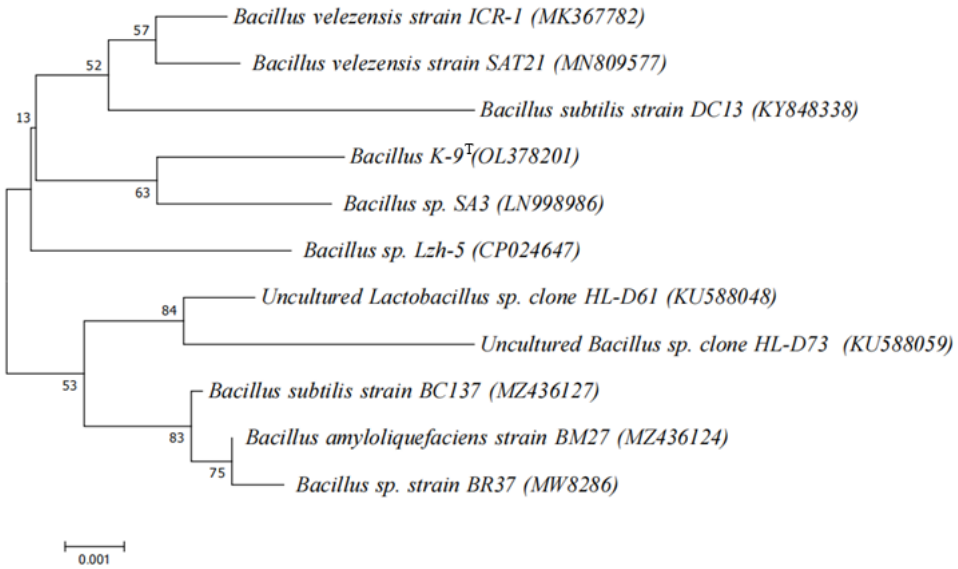


**Figure 2**

Phylogenetic tree of strain K-9 constructed based on 16S rDNA gene sequence Bootstrap values (%) presented at the branches were calculated from 1000 replications. The scale bar means 0.1% sequence difference.

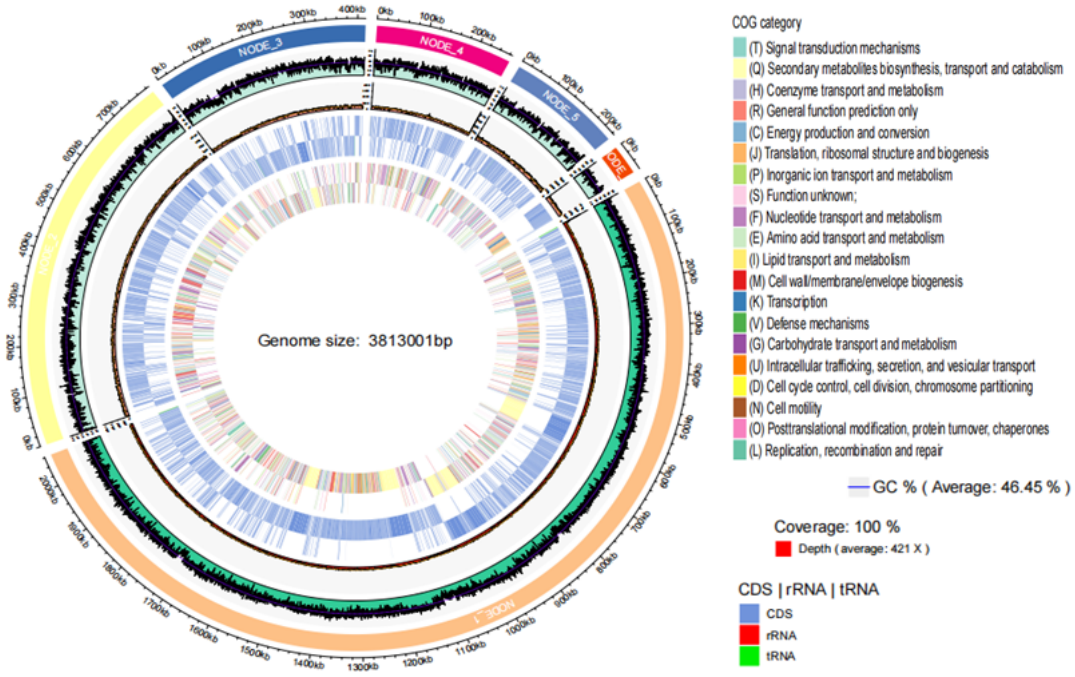


**Figure 3**

CGView circle map of *Bacillus sp.* K-9 genome

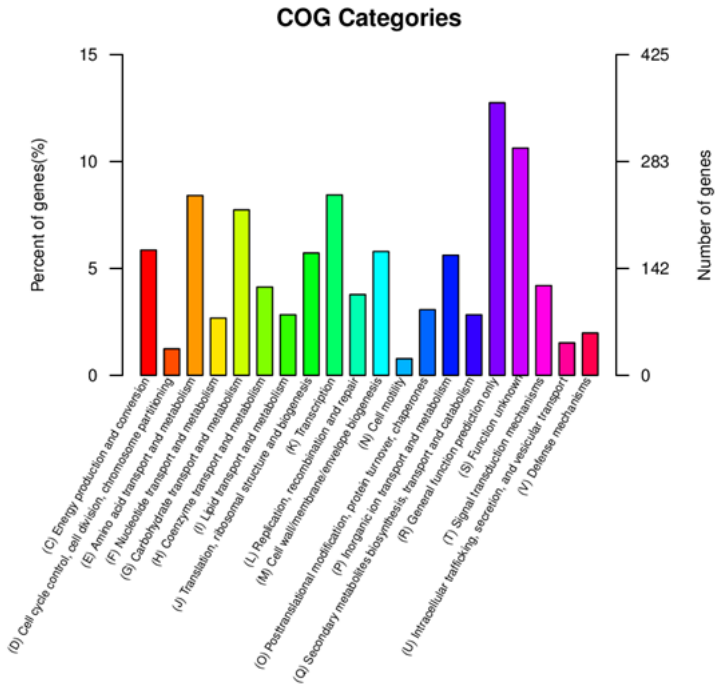


**Figure 4**

COG annotation classification statistics of *Bacillus sp.*K-9 genome

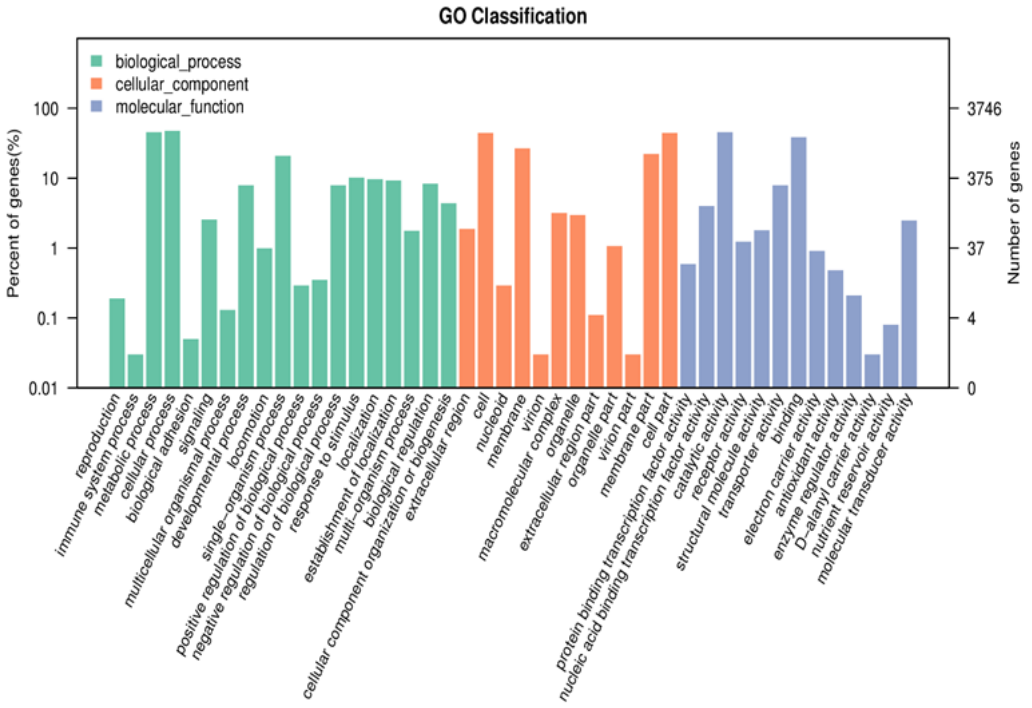


**Figure 5**

Statistics of GO annotation classification of *Bacillus sp.* K-9 genome

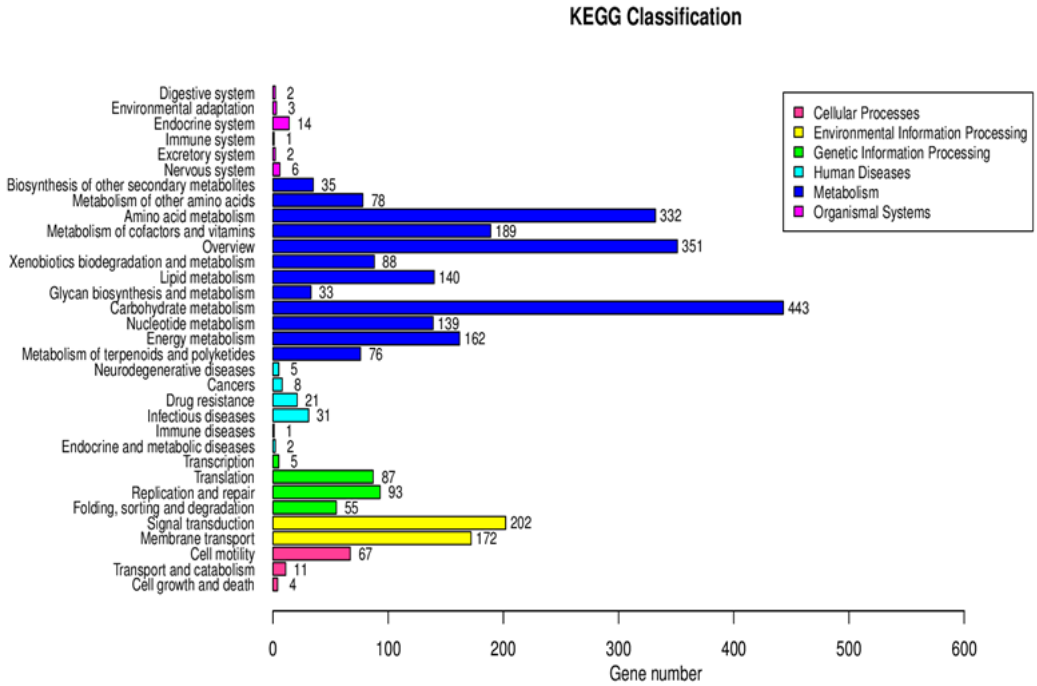


**Figure 6**

Statistics of KEGG annotation classification of *Bacillus sp.*K-9 genome

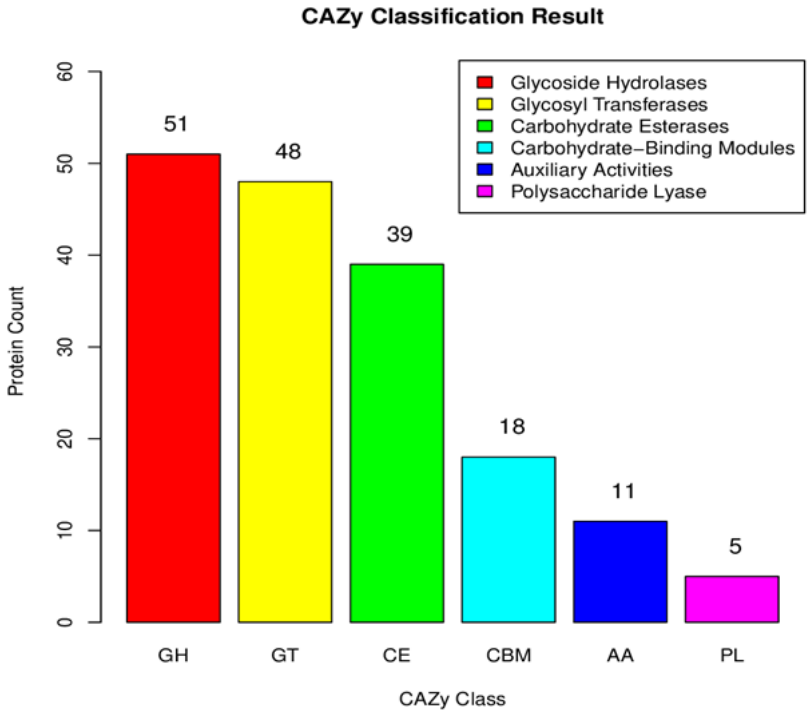


Figure 7

Statistical diagram of CAZy annotation statistics of *Bacillus sp.* K-9 genome

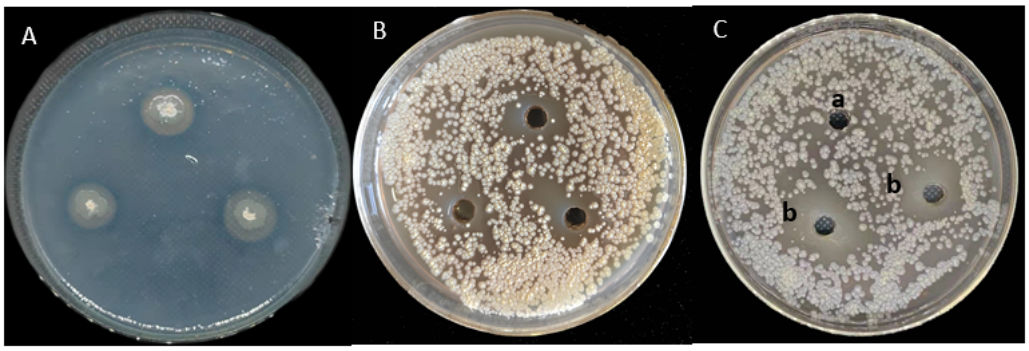


Figure 8

Strain K-9 has ferritin production capacity, inhibition of scab by crude lipopeptide and protein extracts.

A:ferritin production capacity, B:inhibition of scab by crude lipopeptide, C: inhibition of scab by protein extracts, a: PBS buffer solution b:protein extracts.