

Bioactivity and genome analysis of *Bacillus amyloliquefaciens* GL18 isolated from the rhizosphere of *Kobresia myosuroides* in an alpine meadow

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Abstract

The unique eco-environment of the Qinghai–Tibet Plateau breeds abundant microbial resources. In this research, the antagonistic activity, bacteriostatic hydrolase activity, and low temperature, salt, and drought resistance of *Bacillus amyloliquefaciens* GL18, isolated from the rhizosphere of *Kobresia myosuroides* from an alpine meadow, were determined and analysed. The seedlings of *Avena sativa* were root-irrigated using bacteria suspensions (cell concentration $1 \cdot 10^7$ cfu/mL) of GL18, and the growth-promoting effect of GL18 on it was determined under cold, salt and drought stress, respectively. The whole genome of GL18 was sequenced, and its functional genes were analysed. GL18 presented significant antagonistic activity to *Fusarium graminearum*, *Fusarium acuminatum*, *Fusarium oxysporum* and *Aspergillus niger* (inhibition zone diameter > 17 mm). Transparent zones formed on four hydrolase detection media, indicating that GL18 secreted cellulase, protease, pectinase and β -1, 3-glucanase. GL18 tolerated conditions of 10°C, 11% NaCl concentration and 15% PEG-6000 concentration, presenting cold, salt and drought resistance. GL18 improved the cold, salt and drought tolerance of *A. sativa* and it showed significant growth effects under different stress. The total length of the GL18 genome was 3,915,550 bp, and the number of CDS was 3726. Compared with the COG, GO and KEGG databases, 3088, 2869 and 2357 functional genes were annotated, respectively. GL18 contained gene clusters related to antibacterial substances, functional genes related to the synthesis of plant growth-promoting substances, and encoding genes related to stress resistance. This study identified an excellent *Bacillus* strain and provided a theoretical basis for improving stress resistance and promoting the growth of herbages under abiotic stress.

Introduction

Plant growth-promoting rhizobacteria (PGPR), which exist in the rhizosphere and root surface of plants, help plants absorb various nutrients from the environment and promote plant growth directly. They can also promote plant growth by inhibiting pathogenic bacteria or indirectly improving adverse environments, such as salinity, drought, low temperature and heavy metals [1]. As a kind of PGPR, *Bacillus* can promote the growth and development of plants and increase the yield and quality of plants directly by synthesizing auxin, gibberellin, cytokinin and other growth hormone-like chemicals [2]. Zhang et al. [3] found that *Bacillus subtilis* B931, with the activities of secreting antibiotics and plant hormones, which were used to promote the growth of *Dioscorea esculenta*, significantly increased the number of roots and root length. Simultaneously, *Bacillus* showed an antagonistic effect on pathogenic microorganisms, which can secrete some enzymes or active proteins that inhibit plant pathogens in their growth process, reduce the occurrence of plant diseases, and indirectly promote plant growth [4]. Xing et al. [5] found that *B. subtilis* T2 with a high-yield protease can inhibit spore germination and mycelium growth of *Fusarium oxysporum* f. sp. *vasinfectum*. It has been speculated that the protease produced by *Bacillus* T2 can degrade the protein in the cell walls of bacteria, resulting in cell wall defects.

In addition, *Bacillus* can promote plants to absorb a variety of mineral elements, such as organic nitrogen, which can be provided to soil by biological nitrogen fixation, from the environment to improve plant

growth. It can not only promote the circulation of N elements in soil and improve soil fertility but also provide nitrogen elements and promote the absorption and utilization of N to plants by colonization in the root system [6]. Studies have found that fermentation broth of *B. amyloliquefaciens* Y2 can promote the absorption of nitrogen, phosphorus and potassium nutrients in *Zea mays* seedlings, further promoting the growth of plants and significantly increasing the total nitrogen, phosphorus and potassium content [7]. Concurrently, studies have reported that most phosphorus-solubilizing microorganisms are *Bacillus*, which can increase the content of available phosphorus in soil by promoting the transformation of insoluble phosphorus into available phosphorus and improving the utilization rate of phosphorus resources; thus, it is beneficial to plant growth [8].

The special environment of the Qinghai–Tibet Plateau, such as cold, drought, and saline-alkali and anoxic soil, breeds special adaptive microbial resources. *Bacillus* acts as a soil microorganism by forming endophytic spores and has better tolerance and adaptability to special plateau habitats with strong resistance to salt and alkali, cold, electromagnetic radiation, drought, ultraviolet and other adversities [9]. Wu et al. [10] found that psychrophilic *Bacillus* isolated from the Qinghai–Tibet Plateau can promote winter wheat seedlings growth at low temperatures and can be used to produce biological agents. Shao et al. [11] found that after *B. subtilis* NCD-2 interacted with *Lycopersicon esculentum* seedlings under salt stress, it increased the activities of stress-related enzymes and ABA content in seedlings, as well as increased the population number of beneficial microorganisms in the rhizosphere, which indicated that NCD-2 significantly promoted growth and development by improving the tolerance of *Lycopersicon esculentum* under salt stress. Su et al. [12] found that *B. subtilis* QM3 induced the upregulation of drought responsive genes *RD29B*, *RAB18*, *RD20* and *NCED3* by irrigated *Arabidopsis thaliana* with the diluent of QM3 under drought stress, indicating that QM3 enhanced drought tolerance and significantly promoted plant growth. *Avena sativa* is excellent forage suitable for planting and cultivation in the Qinghai–Tibet Plateau and it is an annual herbaceous plant of *Poaceae* [13]. Its stems and leaves are tender and juicy, with good palatability and high nutritional value, and silage or hay can be used as livestock feed, simultaneously, the root system is developed, which can reduce ineffective evaporation and surface runoff, is an important cultivated herbage for protecting ecological environment and preventing soil and water loss in Qinghai-Tibet Plateau [14]. However, it has weak adaptability to abiotic stress during the seedling period, resulting in a yield reduction [15]. Therefore, using biocontrol *Bacillus* with strong resistance to interact with herbage seedlings under abiotic stress is an effective method for vegetation restoration and animal husbandry development in alpine areas by enhancing herbage resistance and promoting herbage growth.

This study determined the antagonistic activity, bacteriostatic-related hydrolase activity, and stress resistance of *Bacillus* GL18 isolated from the rhizosphere of *Kobresia myosuroides* in an alpine meadow of Nianbaoyuze in Qinghai Province. *Avena sativa* seedlings under cold, salt and drought stress interacted with GL18 bacteria suspension, and its promoting effects on herbage under abiotic stress were observed. The whole genome of GL18 was sequenced using third-generation sequencing technology, and the basic characteristics and functional genes were analysed. We screened out *Bacillus* resources with strong stress resistance and good growth-promoting effects adapted to the habitat of the Qinghai

Plateau to provide high-quality bacteria resources and a theoretical basis for bacterial manure development, herbage growth promotion and biomass improvement.

Experimental

Materials. *Bacillus amyloliquefaciens* GL18 was isolated from the *Kobresia myosuroides* rhizosphere from an alpine meadow of Nianbaoyuze in Qinghai Province. *Fusarium graminearum*, *Fusarium acuminatum*, *Fusarium oxysporum* and *Aspergillus niger* were stored in the Key Laboratory of Plateau Grassland Resources and Ecology, Qinghai University. *Avena sativa* cultivar 'Qingyan 1' were provided by the Key Laboratory of Superior Forage Germplasm in the Qinghai–Tibetan Plateau. LB medium was used for *Bacillus* culture, and PDA medium was used for pathogenic fungi culture, in addition to CMC-Na screening medium, protease detection medium, pectinase detection medium, and β -1, 3-glucanase detection medium.

Determination of the antagonistic activity of GL18 to pathogenic fungi. *Fusarium graminearum*, *Fusarium acuminatum*, *Fusarium oxysporum* and *Aspergillus niger* were used to inoculate the center of the PDA plate and cultured to 1/2 of the PDA plate in an incubator at 28°C. A filter paper disc (4 mm in diameter) was then placed into each of the four symmetric points in the center of the plate at 2.5 cm. GL18 was used to inoculate 5 mL of LB liquid medium and incubated at 37°C and 200 r/min for 14 h. Then, 5 μ L of bacterial solution was pipetted into small circles of filter paper on PDA plates with pathogenic fungi. The plates were cultured in an incubator for 2–3 d. The growth of pathogenic fungi without inoculation with GL18 bacterial solution was used as a control. The diameter of the inhibition zone was measured, and the inhibition rate was calculated as follows: Inhibition rate = $[(D - d) / D \cdot 100\%]$, where D is the colony diameter of the control and d is the colony diameter of the GL18 treatment [16].

Determination of the bacteriostatic-related hydrolase activity of GL18. The activities of four hydrolases (cellulase, protease, pectinase, and β -1, 3-glucanase) related to the antagonistic bacteriostatic effects of GL18 were determined. GL18 was used to inoculate 5 mL of LB liquid medium and incubated at 37°C and 200 r/min for 14 h. Five microliters of bacterial solution was then used to inoculate the center of four detection plates and incubated in an incubator at 28°C for 3 d. Gram's iodine solution was injected into a pectinase detection medium and CMC-Na medium and stood for 10 min, and the dye liquor was poured out. A transparent circle indicated that the strain could generate pectinase or cellulase. A protease detection medium was observed directly, regardless of whether a clear transparent zone was generated. A 0.1% Congo red solution was injected into the β -1, 3-glucanase detection medium and stood for 20 min, and the dye liquor was poured out. If there was a transparent zone, it indicated that the strain could generate β -1, 3-glucanase [17].

Determination of the cold resistance of GL18. GL18 was used to inoculate 5 mL of LB liquid medium and incubated at 37°C and 200 r/min for 14 h; 10 μ L of bacterial solution was then pipetted on an LB solid medium plate and spread. Four temperature gradients were set at 4, 10, 14 and 18°C for 5 d, with one treatment repeated 3 times, and its growth was observed and recorded daily [18].

Determination of the salt resistance of GL18. Two hundred microliters of GL18 bacterial solution was pipetted and used to inoculate 5 mL of LB liquid media with NaCl concentrations of 0, 2, 4, 6, 8, 10 and 12% and then oscillated to the logarithmic phase at 37°C and 120 r/min (OD₆₀₀ was about 1.0). The absorbance value was measured at OD₆₀₀ [19].

Determination of drought resistance of GL18. LB liquid media with PEG-6000 concentrations of 0, 3, 6, 9, 12, 15 and 18% were prepared, and 200 µL of GL18 bacterial solution was used to inoculate 5 mL of media. The culture was oscillated to the logarithmic stage (OD₆₀₀ was about 1.0) at 37°C and 120 r/min, and the absorbance was measured at OD₆₀₀ [20].

Suspension preparation. GL18 was used to inoculate 100 mL of LB liquid medium and shaken at 37°C and 200 r/min for 12 h. Then, the supernatant was discarded, and the precipitate was washed twice with sterile water immediately after centrifugation at 8000 *g* and 4°C. The precipitate was mixed with sterile water to obtain a bacterial suspension, and the concentration was adjusted to the corresponding experimental concentration of $1 \cdot 10^7$ cfu/mL (OD₆₀₀ = 1.0).

Sowing. *Avena sativa* seeds with intact seed coats were selected and soaked in 20% sodium hypochlorite solution for 20 min. The disinfectant remaining on the surface was removed with sterile water. The mixture of natural soil and vermiculite (2:1) was sterilized at 120°C. The sterilized seeds were planted in flowerpots with 10 seeds per pot and cultured in a light incubator (26°C, photoperiod 16/8 h) [21].

Growth-promoting effect of GL18 on *Avena sativa* seedlings under low-temperature stress. When the plant height reached 3–5 cm, the temperature of the incubator was adjusted to 10°C. The following two treatments were set up: CK1 (sterile water control) and TB (root irrigation with GL18 bacterial suspension). Thirty milliliters of GL18 suspension (cell concentration 1×10^7 cfu/mL) was used for root irrigation of *Avena sativa* seedlings and the control was treated with 30 mL of sterile water, and the seedlings were irrigated twice (once every 2 d). After root irrigation, the seedlings were carefully removed from the soil, and the roots were washed with sterile water. Plant height and root length were measured, and fresh weight was recorded after removing surface moisture with blotting paper.

Growth-promoting effect of GL18 on *Avena sativa* seedlings under salt stress. When the plant height reached 3–5 cm, two treatments were set up: CK2 (sterile water control) and SB (root irrigation with GL18 bacterial suspension). A total of 30 mL of GL18 suspension (cell concentration 1×10^7 cfu/mL) was used for root irrigation of *Avena sativa* seedlings, and the control was treated with 30 mL of sterile water. The seedlings were irrigated twice (once every 2 d). After root irrigation with GL18, 30 mL of 11% NaCl solution was used to induce salt stress. After 2 d, the seedlings were carefully removed from the pot, and the roots were washed with sterile water. Plant height and root length were measured, and fresh weight was recorded after removing surface moisture with blotting paper.

Growth-promoting effect of GL18 on *Avena sativa* seedlings under drought stress. When the plant height reached 3–5 cm, two treatments were set up: CK3 (sterile water control) and DB (root irrigation with GL18

bacterial suspension). A total of 30 mL of GL18 suspension (cell concentration 1×10^7 cfu/mL) was used to irrigate the roots of *Avena sativa* seedlings, and the control was treated with 30 mL of sterile water. The seedlings were irrigated twice (once every 2 d). After root irrigation with GL18, 30 mL of 15% PEG-6000 solution was used to induce drought stress. After 2 d, the seedlings were carefully removed from the pot, and the roots were washed with sterile water. Plant height and root length were measured, and fresh weight was recorded after removing surface moisture with blotting paper.

Whole genome sequencing of GL18. GL18 was used to inoculate 80 mL of LB liquid medium and was oscillated at 37°C and 200 r/min for 14 h. Samples were then centrifuged for 10 min at 8000 *g* to collect the precipitate. After being frozen with liquid nitrogen, the samples were placed into an incubator with drikold. The sample was sent to the Shanghai Majorbio Technology Limited Company (Shanghai, China) for whole-genome sequencing.

GL18 genome assembly and prediction. The assembly software SOAPdenovo2 [22] was used to concatenate multiple K-mer parameters of the optimized sequence after second-generation sequencing to obtain the optimal contigs assembly, and the assembly software Unicycler [23] was used for the third-generation sequence assembly. Glimmer [24], GeneMarkS [25] and Prodigal (<https://github.com/hyattpd/Prodigal>) software were used to forecast the coding sequence of the genome. tRNAscan-SE v2.0 [26] and Barnap software (<https://github.com/tseemann/barnap>) were used to predict the tRNA and rRNA contained in the genome. Circos software [27] was used to draw the genome circle map of strain GL18.

Functional annotation of the GL18 gene. Diamond (<https://github.com/bbuchfink/diamond>) [28] was used to make comparisons in the EggNOG and KEGG databases [29]. The software blast2go (<https://www.blast2go.com/>) was used to make comparisons in the GO database (<http://www.geneontology.org/>) and to obtain the functional annotation information to analyze functional genes of GL18.

Results

Antagonistic activity of GL18 to pathogenic fungi. The antagonistic activity test showed that GL18 inhibited the growth and expansion of *F. graminearum*, *F. acuminatum*, *F. oxysporum* and *A. niger* to varying degrees. The average diameters of the inhibition zones were 20, 24, 18 and 21 mm, respectively; the inhibition rates to the four pathogenic fungi ranged from 40 to 54%. Among them, GL18 had the most significant inhibitory effect on *F. acuminatum* with an inhibitory rate of 53.3%, and the inhibitory rates on *F. graminearum*, *F. oxysporum* and *A. niger* were 44.4, 40.0 and 46.7%, respectively, which significantly inhibited the growth and expansion of mycelia and showed strong antagonistic activity (Fig. 1a, Table 1).

Bacteriostatic-related hydrolase activity of GL18. The activities of cellulase, protease, pectinase and β -1, 3-glucanase secreted by GL18 were detected by hydrolase detection media. The transparent zones indicated that the strain could produce antibacterial hydrolase, and the size of the transparent zone

indicated its enzyme production ability. GL18 formed a transparent zone on different hydrolase detection media, indicating that it secreted different hydrolases of cellulase, protease, pectinase and β -1, 3-glucanase, which degraded the cell walls of the pathogens and inhibited their growth. The average diameters of the transparent zones on the detection media for cellulase, protease, pectinase and β -1, 3-glucanase were 42, 54, 38 and 32 mm, respectively, showing strong cellulase and protease production ability. It was speculated that GL18 could inhibit growth and reduce the activity of pathogenic bacteria by degrading the cell wall of pathogenic bacteria, thus antagonizing plant pathogens (Fig. 1b, Table 1).

Table 1
Plant growth-promoting traits of strain GL18

Test	Antagonistic to fungal pathogens			
	<i>F. graminearum</i>	<i>F. acuminatum</i>	<i>F. oxysporium</i>	<i>Aspergillus niger</i>
Qualitative	+++	++++	++	+++
Inhibition zone (mm)	20 $\pm$ 1.500c	24 $\pm$ 2.179ab	18 $\pm$ 1.000c	21 $\pm$ 1.323bc
Antibacterial ratio	44.4%	53.3%	40.0%	46.7%
Test	Ectoenzyme			
	Cellulase	Protease	Pectinase	β 1, 3glucanase
Qualitative	+++	++++	+++	++
Transparent zone (mm)	42 $\pm$ 2.000b	54 $\pm$ 2.500a	38 $\pm$ 2.000b	32 $\pm$ 1.000c

$\pm$ means standard deviation, different letters indicate a significant difference at the level of 0.05 ($P < 0.05$).

Low-temperature resistance. At 14 and 18°C, strain GL18 grew and formed a colony after 1 d. At 10°C, it grew and expanded quickly, forming a colony after 2 d. At 4°C, GL18 could hardly grow, therefore, GL18 has a certain tolerance to a low-temperature environment.

Salt resistance. The concentration of bacterial liquid is usually measured by absorbance at OD₆₀₀, which reflects the relative salt tolerance of the strain under salt stress. The OD₆₀₀ value of GL18 in the 2% NaCl concentration was higher than that in the control, indicating that the 2% NaCl concentration was beneficial to GL18 growth. With the increase in NaCl concentration, the OD₆₀₀ value of GL18 showed a downward trend. The concentration of bacterial liquid decreased, indicating that the growth of GL18 was gradually inhibited. When the NaCl concentration reached 11%, a small amount of GL18 survived but grew slowly. When the NaCl concentration reached 12%, GL18 could not survive. Therefore, GL18 grew at a NaCl concentration between 2% and 11% and had strong salt tolerance (Fig. 2a).

Drought resistance. The drought resistance of GL18 was determined by simulating drought stress with different PEG-6000 concentrations. GL18 was not seriously affected by drought stress when the PEG-6000 concentration was below 9%. With the increase in the PEG-6000 concentration, the OD₆₀₀ value of GL18 decreased, and the bacterial concentration of the strain liquid decreased, indicating that the growth of GL18 was affected to a certain extent. The maximum PEG-6000 concentration that GL18 could tolerate was 15%. GL18 had strong drought tolerance and a certain drought tolerance to arid habitats (Fig. 2b).

Growth-promoting effect on *Avena sativa* under cold stress. A GL18 bacteria suspension (cell concentration 1×10^7 cfu/mL) was used to irrigate the roots of *Avena sativa* seedlings twice at 10°C, and plant height, root length and fresh weight increased significantly compared to CK1 (Fig. 3a). The average plant height and root length of the seedlings were 8.03 and 4.46 cm, respectively, increasing by 51.80% and 38.94%, compared with CK1 (Fig. 3b). The average fresh weight was 0.11 g, with an increase of 74.04% (Fig. 3c). Strain GL18 had a significant growth-promoting effect on *Avena sativa* seedlings under cold stress.

Growth-promoting effect on *Avena sativa* under salt stress. *Avena sativa* seedlings were irrigated twice with a GL18 bacteria suspension (cell concentration 1×10^7 cfu/mL) and then irrigated with 30 mL of 11% NaCl solution. On the second day after salt stress, plant height, root length and fresh weight increased significantly compared with CK2 (Fig. 3a). The average plant height and root length of the *Avena sativa* seedlings were 6.89 and 2.97 cm, respectively, increasing by 22.82% and 45.59%, respectively, compared with CK2 (Fig. 3b). The average fresh weight was 0.05 g, with an increase of 70.07% (Fig. 3c). GL18 had a significant growth-promoting effect on *Avena sativa* seedlings under salt stress.

Growth-promoting effect on *Avena sativa* under drought stress. *Avena sativa* seedlings were irrigated twice with the GL18 bacteria suspension (cell concentration 1×10^7 cfu/mL) and then irrigated with 30 mL of 15% PEG-6000 solution. On the second day after drought stress, plant height, root length and fresh weight increased significantly compared with CK3 (Fig. 3a). The average plant height and root length of the *Avena sativa* seedlings were 10.44 and 3.49 cm, respectively, increasing by 39.39% and 57.74%, compared with CK3 (Fig. 3b). The average fresh weight was 0.12 g, with an increase of 12.5% (Fig. 3c). GL18 had a significant growth-promoting effect on *Avena sativa* seedlings under drought stress.

Basic features of the genome. According to the analysis of assembly results, the genome of GL18 was composed of a chromosome with a DNA sequence of 3,915,550 bp, with 272,699 reads, a total base length of 2,249,346,685 and a total base number of 1,276,942,372. The GC content was 46.47%, the number of CDS was 3726, and the total length of coding genes was 3,462,321 bp, with an average length of 929.23 bp. GL18 contained 27 rRNA, comprising nine 16S rRNA, nine 23S rRNA and nine 5S rRNA. The number of tandem repeats was 38, and the total length was 7570. The accession number of the GL18 genome sequence in NCBI is CP096033, and Fig. 4 shows the genome circle diagram.

Functional gene annotation of GL18. COG annotation results indicated that the genome sequence of GL18 was compared with the eggNOG database, and COG function annotation was performed. A total of 3088 genes were annotated, accounting for 82.88% of all genes. Among them, the known predicted functional genes were more related to amino acid transport and metabolism, transcription, carbohydrate transport and metabolism, general function prediction, translation, ribosomal structure, and biogenesis (Fig. 5a).

GO annotation results indicated that the genome sequence of GL18 was compared with the gene ontology database to perform GO gene annotation. A total of 2869 functional genes were annotated, accounting for 77% of all genes, which were divided into biological process (BP), cellular component (CC) and molecular function (MF). Among them, 1411 genes related to biological processes were mainly involved in the regulation of transcription and DNA-templated, translation, carbohydrate metabolic process, and transmembrane transport, as well as in the biosynthetic process of fatty acid, amino acid transport and other processes. In cellular components, 1383 functional genes were closely related to integral components of membrane, plasma membrane, cytoplasm and ribosome. In molecular function, 2319 functional genes were mainly associated with ATP binding, DNA binding, metal ion binding, hydrolase activity and transmembrane transporter activity (Fig. 5b).

KEGG annotation results indicated that the genome sequence of GL18 was annotated with related genes of the pathway in the KEGG database. A total of 2357 genes were found to be involved in functional annotation, including 174 pathways involved in cellular processes, 3033 pathways involved in metabolism, 215 pathways involved in genetic information processing, and 302 pathways involved in environmental information processing, which were mainly related to carbohydrate metabolism, amino acid metabolism, and metabolism of cofactors and vitamins (Fig. 5c).

Functional genes related to antagonistic activity. AntiSMASH software [30] was used to predict the gene cluster of secondary metabolite synthesis of GL18, and 13 gene clusters of secondary metabolites were predicted (Table 2). Five types of substances with antibacterial activity were related to non-ribosomal peptide synthase or polyketone synthase: surfactin, bacillaene, fengycin, iturin and bacillibactin. Cluster 1 was 91% similar to the known gene cluster and contained 41 coding genes, including *srfAA*, *srfAB*, *srfAC* and *srfATE*, which encode surfactin synthesis. Surfactin is a lipopeptide biosurfactant with antibacterial, antiviral and anti-mycoplasma activities [31]. Cluster 6 was 100% similar to the known gene cluster and contained 52 coding genes, which contain genes of the *pks* family encoding bacillaene synthesis, including *pksC*, *pksE*, *pksG*, *pksI*, *pksJ*, *pksH*, *pksL*, *pksM*, *pksN* and *pksR*. Bacillaene is a polyketone antibiotic that can effectively inhibit the growth of pathogenic bacteria [32]. Bacillibactin can also absorb and accumulate iron ions under iron-limited conditions and compete with other microorganisms to exert its antibacterial effect [33]. Cluster 7 was found to be 100% similar to a known gene cluster containing 64 coding genes, including *ituA*, *ituB* and *ituC*, which encode the key genes of iturin synthesis, and *ppsA*, *ppsB*, *ppsC*, *ppsD* and *ppsE*, which encode the key genes of fengycin synthesis. Iturin is a non-ribosomal synthesized cyclolipidic peptide that can inhibit fungal activity by changing the permeability of the fungal cell membrane [34]. Fengycin is a circular lipopeptide that acts as an antagonist against fungi by

damaging the cell membranes of the target cells [35]. Cluster 12 was found to be 100% similar to the known gene cluster and contained 45 coding genes, including *dhbF*, a key gene involved in non-ribosomal peptide synthase. Cluster 13 was found to be 100% similar to the bacilysin gene cluster and contained 43 coding genes.

In addition, clusters 4, 8, 9 and 11 with unknown functions were found in the genome of GL18, including two terpene, one T3PKS and one NRPS, speculating that the GL18 genome may have gene clusters of synthesizing other antibacterial substances. Cluster 3 was only 7% similar to the known gene cluster and contained 41 coding genes, suggesting that the gene cluster might synthesize new antibacterial substances (Fig. 6). Therefore, we speculated that *Bacillus* GL18 could promote plant growth by synthesizing antibacterial substances to inhibit the growth of pathogens indirectly through the expression of the functional genes of bacteriostatic-related substances.

Table 2
Annotation statistics of secondary metabolites

Cluster ID	Type	Start	End	Similar Cluster	Similarity (%)	Gene No.
cluster1	NRPS	318207	383067	surfactin	91	41
cluster2	LAP	718153	740336	plantazolicin	91	19
cluster3	PKS-like	935681	976926	butirosin A / butirosin B	7	41
cluster4	terpene	1062550	1079780	–	–	22
cluster5	transAT-PKS	1366839	1454621	macrolactin H	100	43
cluster6	transAT-PKS	1676753	1786365	bacillaene	100	52
cluster7	NRPS	1846486	1983924	fengycin	100	64
cluster8	terpene	2010878	2032762	–	–	22
cluster9	T3PKS	2099247	2140348	–	–	47
cluster10	transAT-PKS- like	2256753	2362930	difficidin	100	59
cluster11	NRPS	2845913	2900807	–	–	43
cluster12	NRPS	2997798	3049590	bacillibactin	100	45
cluster13	other	3572815	3614234	bacilysin	100	43
– means none						

Functional genes related to growth promotion. Key genes of *trpC*, *trpB* and *trpA* encoding proteins related to the synthesis of growth hormones exist in the GL18 genome, which are involved in the tryptophan-dependent IAA synthesis pathway. It is speculated that GL18 can promote plant growth directly by synthesizing IAA. Moreover, the functional genes of *miaB* and *idi* are related to cytokinin synthesis in the GL18 genome. Cytokinin is involved in plant growth and development, thus improving the tolerance of plants to abiotic stress [36]. In addition, the GL18 genome contains a variety of functional genes related to the growth promotion of *Bacillus*, including *glnB* [37], *trkH*, *trkA* and *kch* [38], which are involved in the synthesis of nitrogen or potassium required for plant growth and development. GL18 may improve the soil environment and promote plant growth through nitrogen fixation and phosphorus hydrolysis. Functional genes *YclQ* and *fetB* are related to the synthesis and transport of siderophores [39], which can improve the ferritin content of plants through iron chelation and indirectly promote plant growth (Table 3).

Functional genes related to stress tolerance. The GL18 genome contains gene clusters encoding key genes related to inducing plant resistance, in which functional genes *lytE*, *ykfC* and *cwlO* are related to ABA biosynthesis [40]. ABA induces plant resistance by regulating the physiological response process of plants to abiotic stress. The GL18 genome also contains genes that encode *pheT*, *pheS* and *menF*, which are key genes involved in SA biosynthesis, and SA can respond to the antioxidant defense system of plants and improve the stress resistance of plants [41]. Meanwhile, the GL18 genome has functional genes encoding osmotic regulatory substances involved in response to abiotic stress. Gene clusters of the encoding Na^+/H^+ antiporter, including *mnhA*, *mnhB*, *mnhC*, *mnhD*, *mnhE*, *mnhF* and *mnhG* [42], can regulate intracellular pH, maintain ion homeostasis and promote cell growth under stress. It also has genes *proV*, *proW* and *opuA* encoding osmoprotein synthesis of proline and betaine [43], which can increase the tolerance to osmotic stress. In addition, the GL18 genome also contains the key gene *cspA* encoding cold shock proteins CspC, CspB and CspD [44], which participate in the low-temperature response, maintain the normal growth and metabolism of cells, and enhance the low-temperature resistance of cells (Table 3).

Table 3
Related function gene analysis of GL18

Gene name	Location	Length	Function
<i>trpC</i>	2 178 057 – 2 177 305	753	Indole-3-glycerol phosphate synthase, involved in the tryptophan-dependent IAA synthesis pathway, associated with defense responses
<i>trpA</i>	2175471 – 2174674	798	Tryptophan synthase subunit alpha gene
<i>trpB</i>	2176666 – 2175464	1 203	Tryptophan synthase subunit beta gene
<i>miaB</i>	1 686 372 – 1 687 901	1 530	Isopentenyl adenine gene, considered as the main cytokinin
<i>idi</i>	2 196 713 – 2 195 664	1 050	Isopentenyl adenine gene, considered as the main cytokinin
<i>glnB</i>	3 483 211 – 3 483 561	351	Involved in the regulation of nitrogen fixation
<i>trkH</i>	1 283 441–1 284 793	1 353	TrkH family potassium uptake protein, involved in absorption and utilization of K ⁺
<i>trkA</i>	1 378 966 – 1 379 631	666	Ktr system potassium transporter gene KtrC
<i>kch</i>	2 956 934 – 2 957 920	987	Potassium channel family protein gene
<i>yclQ</i>	401 740 – 402 684	945	Siderophore synthase gene, involved in growth-promoting gene expression
<i>fetB</i>	1 197 756 – 1 196 995	762	Ferrous iron transport protein B
<i>lytE</i>	942 569 – 943 393	825	ABA biosynthesis gene, induced stress resistance
<i>ykfC</i>	1 243 194 – 1 244 093	900	ABA biosynthesis gene, induced stress resistance
<i>cwlO</i>	3 314 292 – 3 312 880	1 413	ABA biosynthesis gene, induced stress resistance

Gene name	Location	Length	Function
<i>pheT</i>	2 690 097 – 2 687 683	2 415	SA biosynthesis gene, induced stress resistance
<i>pheS</i>	2 691 145 – 2 690 111	1 035	SA biosynthesis gene, induced stress resistance
<i>menF</i>	2 915 727 – 2 914 312	1 416	SA biosynthesis gene, induced stress resistance
<i>mnhA</i>	2 984 830 – 2 987 232	2 403	Na ⁺ /H ⁺ antiporter subunit A, maintained normal growth under high salt stress
<i>mnhB</i>	2 987 229 – 2 987 660	432	Na ⁽⁺⁾ / H ⁽⁺⁾ antiporter subunit B, maintained normal growth under high salt stress
<i>mnhC</i>	2 987 660 – 2 988 001	342	Na ⁽⁺⁾ / H ⁽⁺⁾ antiporter subunit C, maintained normal growth under high salt stress
<i>mnhD</i>	2 987 985 – 2 989 475	1 491	Monovalent cation/H ⁺ antiporter subunit D, maintained normal growth under high salt stress
<i>mnhE</i>	2 989 481 – 2 989 957	477	Na ⁺ /H ⁺ antiporter subunit E, maintained normal growth under high salt stress
<i>mnhF</i>	2 989 957 – 2 990 241	285	Na ⁽⁺⁾ / H ⁽⁺⁾ antiporter subunit F1, maintained normal growth under high salt stress
<i>mnhG</i>	2 990 225 – 2 990 599	375	Monovalent cation/H ⁽⁺⁾ antiporter subunit G, maintained normal growth under high salt stress
<i>proV</i>	289 900 – 291 156	1 257	Involved in proline or betaine synthesis, alleviated cell apoptosis
<i>proW</i>	291 157 – 292 005	849	Involved in proline or betaine synthesis, alleviated cell apoptosis
<i>opuA</i>	3 214 816 – 3 213 671	1 146	Betaine/proline/choline family ABC transporter ATP-binding protein gene
<i>cspA</i>	516 907 – 517 110	204	Cold shock protein CspC
<i>cspA</i>	909 691 – 909 491	201	Cold shock-like protein CspB

Gene name	Location	Length	Function
<i>cspA</i>	2 110 200 - 2 110 400	201	Cold-shock protein CspD

Discussion

Under the conditions of abiotic stress, such as drought, saline-alkali and low temperatures, plant rhizosphere growth-promoting bacteria (PGPR) can induce stress responsive proteins by regulating the related signal pathways to respond to stress in plants and to alleviate the damage in plants caused by saline-alkali, drought, waterlogging and temperature stress [45]. Therefore, screening *Bacillus* with excellent stress resistance that adapted to special habitats on the Qinghai–Tibet Plateau, utilizing them to promote alpine herbage growth, has a certain practical significance for improving grassland biomass, restoring grassland degradation and promoting the development of animal husbandry.

Studies have shown that *Bacillus* can inhibit the growth of plant pathogens by inducing systemic resistance, producing a variety of antibacterial substances, secreting hydrolase, forming biofilms or competing for ecological niche in the rhizosphere [46]. Yang et al. [37] found that *B. amyloliquefaciens* DGL1 has significant antifungal activity, and it has gene clusters encoding antibacterial, such as *ppsA* and *ppsB*, the key genes related to fengycin synthesis. In this study, *B. amyloliquefaciens* GL18 had significant antagonism effects on the growth of *F. graminearum*, *F. acuminatum*, *F. oxysporum* and *A. niger*. The key genes related to antibacterial activity were found in the gene clusters of secondary metabolites of GL18, encoding surfactin, bacillaene, fengycin, iturin and bacillibactin. GL18 may inhibit the growth of pathogens through the synthesis and secretion of antimicrobial substances to improve plant disease resistance, indirectly promoting plant growth. In addition, Guleria et al. [47] reported that proteases produced by *B. amyloliquefaciens* had biocontrol effects on *F. oxysporum*. In this study, transparent zones were generated after GL18 was used to inoculate the different detection media of bacteriostatic hydrolases, indicating that GL18 produced cellulase, protease, pectinase and β -1, 3-glucanase. It was speculated that GL18 could inhibit the growth of pathogenic fungi by hydrolyzing the main components of the cell wall, which is consistent with the antagonism of *Bacillus* to many pathogenic bacteria.

Bacillus can form resistant spores with strong environmental adaptability, which can improve plant tolerance to abiotic stress and promote plant growth directly or indirectly [48]. Studies have reported that *Bacillus* can enhance plant tolerance and promote plant growth and development by improving the water absorption capacity of plants under stress, enhancing the absorption of essential nutrients, accumulating osmotic substances and other mechanisms [49]. The GL18 genome encodes the functional genes participating in the response to abiotic stress by osmotic regulation substances, such as the gene cluster

of Na^+/H^+ reverse transfer protein and genes related to proline and betaine synthesis, the key genes encoding cold response proteins, and genes involved in the biosynthesis of ABA- and SA-related functional genes. It is speculated that GL18 could promote plant cell growth normally by the mechanism of maintaining ion homeostasis, osmotic regulation, inducing stress protein and improving stress resistance. In addition, GL18 suspension was used for root irrigation of *Avena sativa* seedlings under cold, salt and drought stress. Compared with the control, the plant height, root length and fresh weight of the three herbage were significantly increased under different abiotic stressors, showing a significant growth-promoting effect. Simultaneously, genome analysis indicated that GL18 has a variety of growth-promoting functional genes, including key genes related to auxin and cytokinin synthesis. Studies have shown that IAA secreted from *Bacillus* can affect plant growth, development and stress response by changing the endogenous pool of IAA [50]. In addition, functional genes related to the synthesis of nitrogen and potassium required by the growth and development of plants were found in the GL18 genome. GL18 may provide nutrients for plants, improve the soil environment and promote plant growth through nitrogen fixation and potassium digestion. GL18 also has functional genes related to siderophore synthesis and transport, and studies have shown that siderophores can promote bacterial sporulation and chelate iron to indirectly promote plant growth [51]. In conclusion, GL18 has excellent adaptability to adversities and a growth-promoting effect.

In this study, *B. amyloliquefaciens* GL18 showed significant antifungal activity and secreted four kinds of bacteriostatic hydrolases. Simultaneously, GL18 had a strong tolerance to low-temperature, saline-alkali, and drought stress and promoted the growth of alpine herbage under different abiotic stressors, which showed better adaptability in extreme habitats. According to studies of biological activity and genome sequences of GL18, we analysed the bio-control function, growth-promoting effect and corresponding functional genes to explore the antagonistic activity and growth-promoting mechanism of GL18. The excellent adversity adapting and disease resistance abilities of GL18 were shown, which provides a theoretical basis of antagonistic activity and growth-promotion of GL18 and excellent strains for bio-bacterial manure resources. It is expected to be applied in disease control and growth promotion of alpine herbage.

Declarations

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants as objects of research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Author Y. X. designed the study and administrated the project. L. C. performed the experiments, analysed data, and wrote the draft version of the manuscript. L. C. and X. W. collected samples for whole-genome sequencing and analysed the genome data. L. Wu., J. Y. and Y. Gao. detected the antagonistic activity, bacteriostatic hydrolase activity, and low temperature, salt, and drought resistance. Y. Mi. and F. Yang. detected the growth-promoting effect of *A. sativa*. under cold, salt and drought stress. All authors have read and agreed to the published version of the manuscript.

References

1. Ahmad F., Ahmad I., Khan M. S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Res.* 163, 173-181.
2. Ye J. J., Cao N. N., Wu J. M., Hu Z. Z., Zhang J. F. 2014. Research progress on application of biocontrol *Bacillus*. *Journal of Northwest A & F University* (Natural Science Edition). 42, 185-190.
3. Zhang X., Tang W. h., Zhang L. Q. 2007. Biological control of plant diseases and plant growth promotion by *Bacillus subtilis* B931. *Acta Agronomica Sinica.* 33, 236-241.
4. Xie Y. L., Ma L. Z., Xu Z. W., Zhang Y., Li X. L. 2014. Identification of low-temperature adapting PGPR strains isolated from frozen desert area and their antimicrobial and growth-promoting activity. *Chinese Journal of Biological Control.* 30, 94-100.
5. Xing J. S., Li R., Zhao L., Liang Y. C., Zhu X. P. 2008. Purification, characterization and antagonism of an extracellular protease from *Bacillus subtilis* strain T2. *Acta Phytopathologica Sinica.* 38, 377-381.
6. Panneerselvam P., Senapati A., Kumar U., Sharma L., Lepcha P., Prabhukarthikeyan S. R., Jahan A., Parameshwaran C., Govindharaj G. P. P., Lenka S., Nayak P. K., Mitra D., Sagarika M. S., Thangappan S., Sivakumar U. 2019. Antagonistic and plant-growth promoting novel *Bacillus* species from long-term organic farming soils from Sikkim, India. *3 Biotech.* 9, 416.
7. Cui W. Y., He P. J., Yang L. j., He P. F., He P. b., Wu Y. X., Li X. Y., He Y. Q. 2019. Phosphorus- and potassium-dissolving and nitrogen-fixing capabilities and growth-promotion effect of B9601-Y2 on maize. *Journal of Maize Sciences.* 27, 155-160+168.

8. Jiang W., Wu Q. L., Dou X., Guan Z. B., Cai Y. J., Liao X. R. 2018. Characterization of a phosphorus-solubilizing alkaline phosphatase AP3 cloned from *Bacillus amyloliquefaciens* YP6. *Microbiology China*. 45, 1408-1415.
9. Liu G. H., Lin N. Q., Lin Y. Z., Liu B. 2008. Advances in taxonomy and application of Genus *Bacillus*. *Fujian Journal of Agricultural Sciences*. 23, 92-99.
10. Wu H. J., Gu Q., Xie Y. L., Lou Z. Y., Xue P. Q., Liu F., Yu C. J., Jia D. D., Huang G. C., Zhu B. C., Schneider A., Blom J., Lasch P., Borriss R., Gao X. W. 2019. Cold-adapted *Bacilli* isolated from the Qinghai-Tibetan Plateau are able to promote plant growth in extreme environments. *Environmental Microbiology*. 21, 3505-3526.
11. Shao M. Q., Zhao W. S., Su Z. H., Dong L. H., Guo Q. G., Ma P. 2021. Effect of *Bacillus subtilis* NCD-2 on the growth of tomato and the microbial community structure of rhizosphere soil under salt stress. *Scientia Agricultura Sinica*. 54, 4573-4584.
12. Su X. F. 2022. Effects of *Bacillus subtilis* on drought and salt stress tolerance of *Arabidopsis thaliana*. *Molecular Plant Breeding*. 20, 536-543.
13. Hou L. Y., Zhu Z. Y., Yang J., Ma H., Li Q. M., Tao J., Fan B., Hao J. X., Yan J. Y., Zhou Q. P., Bai W. M. 2019. Current status, problems and potentials of forage oat in China. *Journal of Southwest Minzu University (Natural Science Edition)*. 45(03): 248-253.
14. Gao S., Yang J. S., Yao R. J., Cao Y. F., Tang C. 2020. Effects of soil amelioration measures mitigating soil salinity and improving crop P uptake in coastal area of North Jiangsu. *Acta Pedologica Sinica*. 57(05): 1219-1229.
15. Jin Y. T., Liu W. H., Liu K. Q., Liang G. L., Jia Z. F. 2022. Effect of water deficit stress on the chlorophyll fluorescence parameters of *Avena sativa* 'Qingyan No.1' over the whole crop growth period. *Acta Prataculturae Sinica*. 31, 112-126.
16. Yu C. J., Liu X., Zhang X. Y., Zhang M. X., Gu Y. Y., Ali Q., Mohamed M. S. R., Xu J. H., Shi J. R., Gao X. W., Wu H. J., Gu Q. 2021. Mycosubtilin produced by *Bacillus subtilis* ATCC6633 inhibits growth and mycotoxin biosynthesis of *Fusarium graminearum* and *Fusarium verticillioides*. *Toxins* (Basel). 13, 791.
17. Zubair M., Farzand A., Mumtaz F., Khan A. R., Sheikh T. M. M., Haider M. S., Yu C. J., Wang Y. J., Ayaz M., Gu Q., Gao X. W., Wu H. J. 2021. Novel genetic dysregulations and oxidative damage in *Fusarium graminearum* induced by plant defense eliciting psychrophilic *Bacillus atrophaeus* TS1. *Int J Mol Sci*. 22, 12094.
18. Xie Y. L., Renato D'O., Stefania M., Fan J., Li Y. L. 2017. Molecular identification of cellulose-degrading bio-control *Bacillus* strains and their stress-resistance and growth-promoting characteristics. *Microbiology China*. 44, 348-357.
19. Wu X. H., Wu H. J., Wang R. Y., Wang Z. Q., Zhang Y. M., Gu Q., Farzand A., Yang X., Semenov M., Borriss R., Xie Y. L., Gao X. W. 2021. Genomic features and molecular function of a novel stress-tolerant *Bacillus halotolerans* strain isolated from an extreme environment. *Biology* (Basel). 10, 1030.

20. Xu C. Y., Wang T., Xu T., Su J. Y. 2019. Isolation, screening and identification of a strain of drought resistant bacteria. *Journal of Arid Land Resources and Environment*. 33, 117-123.
21. Ayaz M., Ali Q., Farzand A., Khan A. R., Ling H. L., Gao X. W. 2021. Nematicidal volatiles from *Bacillus atrophaeus* GBSC56 promote growth and stimulate induced systemic resistance in tomato against *Meloidogyne incognita*. *Int J Mol Sci*. 22, 5049.
22. Luo R. B., Liu B. H., Xie Y. L., Li Z. Y., Huang W. H., Yuan J. Y., He G. Z., Chen Y. X., Pan Q., Liu Y. J., Tang J. B., Wu G. X., Zhang H., Shi Y. J., Liu Y., Yu C., Wang B., Lu Y., Han C. L., Cheung D. W., Yiu S. M., Peng S. L., Zhu X. Q., Liu G. M., Liao X. K., Li Y. R., Yang H. M., Wang J., Lam T. W., Wang J. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *GigaScience*. 1, 18.
23. Wick R. R., Judd L. M., Gorrie C. L., Holt K. E. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS comput biol*. 13, e1005595.
24. Delcher A. L., Bratke K. A., Powers E. C., Salzberg S. L. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics*. 23, 673-679.
25. Besemer J., Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. *Nucleic Acids Res*. 33, 451-454.
26. Chan P. P., Lowe T. M. 2019. tRNAscan-SE: Searching for tRNA genes in genomic sequences. *Methods Mol Biol*. 1962, 1-14.
27. Krzywinski M., Schein J., Birol I., Connors J., Gascoyne R., Horsman D., Jones S. J., Marra M. A. 2009. Circos: An information aesthetic for comparative genomics. *Genome Res*. 19, 1639-1645.
28. Jensen L. J., Julien P., Kuhn M., Mering C. V., Muller J., Doerks T., Bork P. 2008. eggNOG: automated construction and annotation of orthologous groups of genes. *Nucleic Acids Res*. 36, 250-254.
29. Kanehisa M., Goto S. 2000. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 28, 27-30.
30. Blin K., Wolf T., Chevrette M. G., Lu X. W., Schwalen C. J., Kautsar S. A., Duran H. G. S., De Los Santos E. L. C., Kim H. U., Nave M., Dickschat J. S., Mitchell D. A., Shelest E., Breitling R., Takano E., Lee S. Y., Weber T., Medema M. H. 2017. antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res*. 45, 36-41.
31. Zhang F., Huo K. Y., Song X. Y., Quan Y. F., Wang S. F., Zhang Z. L., Gao W. X., Yang C. 2020. Engineering of a genome-reduced strain *Bacillus amyloliquefaciens* for enhancing surfactin production. *Microb Cell Fact*. 19, 223.
32. Li H. Y., Han X., Dong Y. J., Xu S. S., Chen C., Feng Y.-ang., Cui Q., Li W. L. 2021. Bacillaenes: decomposition trigger point and biofilm enhancement in *Bacillus*. *ACS Omega*. 6, 1093-1098.
33. Pi H. L., Helmann J. D. 2018. Genome-wide characterization of the fur regulatory network reveals a link between catechol degradation and bacillibactin metabolism in *Bacillus subtilis*. *mBio*. 9, e01451-18.
34. Kim Y. T., Kim S. E., Lee W. J., Fumei Z., Cho M. S., Moon J. S., Oh H. W., Park H. Y., Kim S. U. 2020. Isolation and characterization of a high iturin yielding *Bacillus velezensis* UV mutant with improved

- antifungal activity. *PLoS One*. 15, e0234177.
35. Sur S., Romo T. D., Grossfield A. 2018. Selectivity and mechanism of fengycin, an antimicrobial lipopeptide, from molecular dynamics. *J Phys Chem B*. 122, 2219-2226.
 36. Liu Y., Zhang M. J., Meng Z., Wang B. S., Chen M. 2020. Research progress on the roles of cytokinin in plant response to stress. *Int J Mol Sci*. 21, 6574.
 37. Yang X., Xie Y. L., Chen L., Wu X. H., Wang T., Qing L. T., Chen H. L. 2021. Biological activity of grass growth-promoting and genome analysis of *Bacillus amyloliquefaciens* DGL1 isolated from the rhizosphere of *Nitraria tangutorum* of sand soil in Qinghai Province. *Acta Agrestia Sinica*. 29, 1637-1648.
 38. Levin E. J., Zhou M. Recent progress on the structure and function of the TrkH/KtrB ion channel. 2014. *Curr Opin Struct Biol*. 27, 95-101.
 39. Nicolaou S. A., Fast A. G., Nakamaru-Ogiso E., Papoutsakis E. T. 2013. Overexpression of *fetA* (*ybbL*) and *fetB* (*ybbM*), encoding an iron exporter, enhances resistance to oxidative stress in *Escherichia coli*. *Appl Environ Microbiol*. 79, 7210-7219.
 40. Sassine J., Sousa J., Lalk M., Daniel R. A., Vollmer W. 2020. Cell morphology maintenance in *Bacillus subtilis* through balanced peptidoglycan synthesis and hydrolysis. *Sci Rep*. 10, 17910.
 41. R. Khan M. I., Fatma M., Per T. S., Anjum N. A., Khan N. A. 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front Plant Sci*. 6, 462.
 42. Kashyap D. R., Botero L. M., Lehr C., Hassett D. J., McDermott T. R. 2006. A $\text{Na}^+:\text{H}^+$ antiporter and a molybdate transporter are essential for arsenite oxidation in *Agrobacterium tumefaciens*. *J Bacteriol*. 188, 1577-1584.
 43. Sikkema H. R., Noort M. V. D., Rheinberger J., Boer M. D., Krepel S. T., Schuurman-Wolters G. K., Paulino C., Poolman B. 2020. Gating by ionic strength and safety check by cyclic-di-AMP in the ABC transporter OpuA. *Sci Adv*. 6, eabd7697.
 44. Uppalapati C. K., Gutierrez K. D., Buss-Valley G., Katzif S. 2017. Growth-dependent activity of the cold shock *cspA* promoter + 5' UTR and production of the protein CspA in *Staphylococcus aureus* Newman. *BMC Res Notes*. 10, 232.
 45. Lee S. W., Lee S. H., Balaraju K., Park K. S., Nam K. W., Park J. W., Park K. 2014. Growth promotion and induced disease suppression of four vegetable crops by a selected plant growth-promoting rhizobacteria (PGPR) strain *Bacillus subtilis* 21-1 under two different soil conditions. *Acta Physiologiae Plantarum*. 36, 1353-1362.
 46. Chen M. C., Wang J. P., Liu B., Zhu Y. J., Xiao R. F., Yang W. J., Ge C. P., Chen Z. 2020. Biocontrol of tomato bacterial wilt by the new strain *Bacillus velezensis* FJAT-46737 and its lipopeptides. *BMC Microbiol*. 20, 160.
 47. Guleria S., Walia A., Chauhan A., Shirkot C. K. 2016. Molecular characterization of alkaline protease of *Bacillus amyloliquefaciens* SP1 involved in biocontrol of *Fusarium oxysporum*. *Int J Food Microbiol*. 232, 134-143.

48. Kierul K., Voigt B., Albrecht D., Chen X. H., Carvalhais L. C., Borriss R. 2015. Influence of root exudates on the extracellular proteome of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Microbiology*. 161, 131-147.
49. Ha-Tran D. M., Nguyen T. T. M., Hung S. H., Huang E., Huang C. C. 2021. Roles of plant growth-promoting rhizobacteria (PGPR) in stimulating salinity stress defense in plants: a review. *Int J Mol Sci*. 22, 3154.
50. Spaepen S., Vanderleyden J., Remans R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol Rev*. 31, 425-48.
51. Grandchamp G. M., Caro L., Shank E. A. 2017. Pirated siderophores promote sporulation in *Bacillus subtilis*. *Appl Environ Microbiol*. 83, e03293-16.

Figures

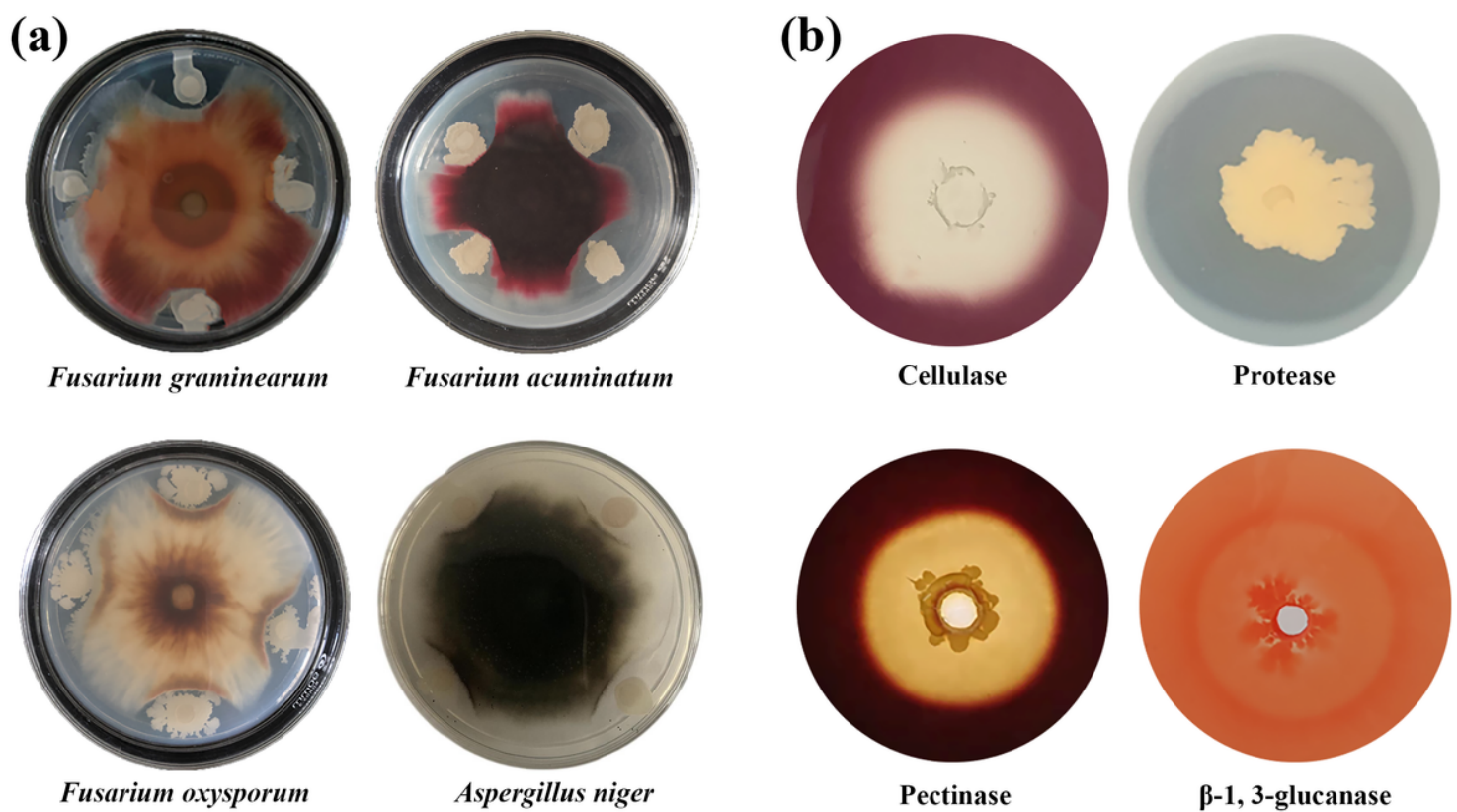


Figure 1

Antagonistic activity of *Bacillus*GL18

(a) Antagonistic activity of *Bacillus* GL18 against the pathogenic fungi. (b) Transparent zones formed by *Bacillus* GL18 on different media.

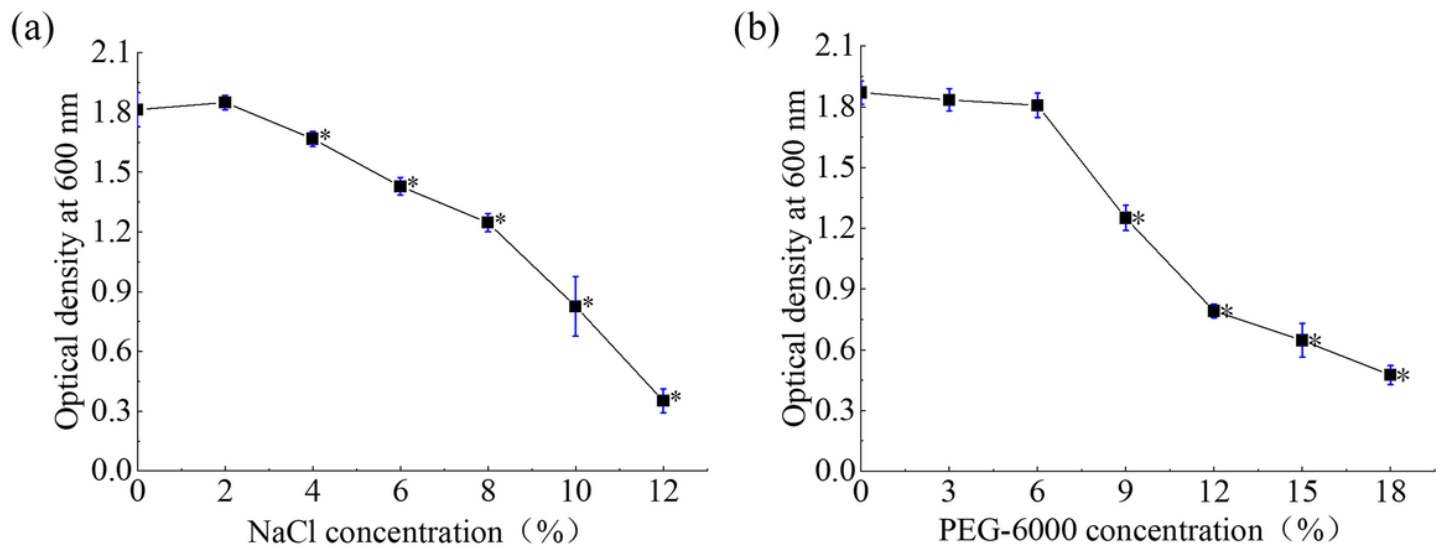


Figure 2

Stress tolerance of strain GL18

(a) Salt resistance of GL18. (b) Drought resistance of GL18.

Note: $\pm$ means standard deviation, *indicate significant difference compared with 0% at the 0.05 level.

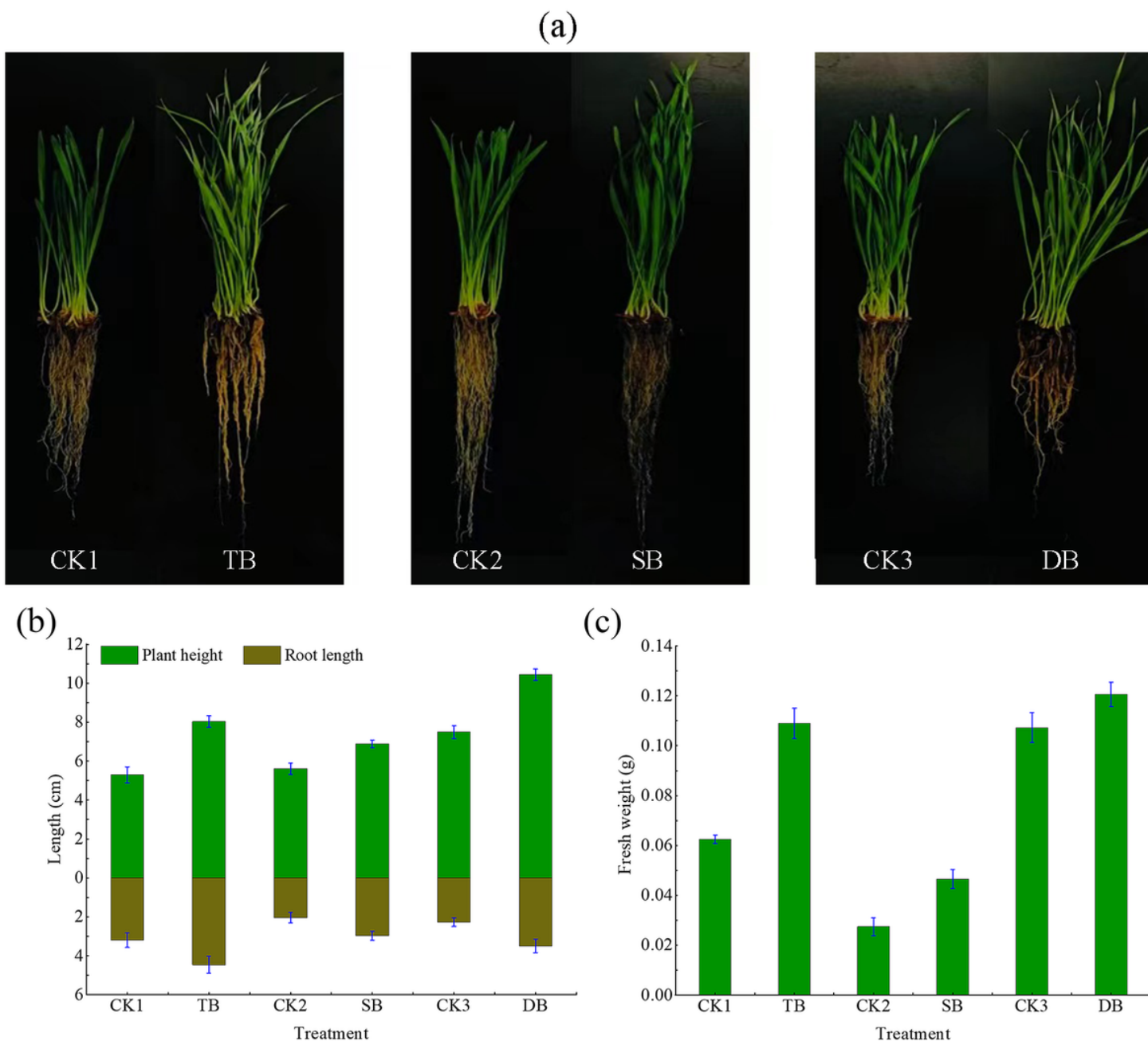


Figure 3

Effects of strain GL18 on the growth of seedlings under stress.

(a) Effects of strain GL18 on the phenotype of seedlings. (b) Effects of strain GL18 on the plant height and root length of seedlings. (c) Effects of strain GL18 on the fresh weight of seedlings. Different letters indicate a significant difference ($P < 0.05$).

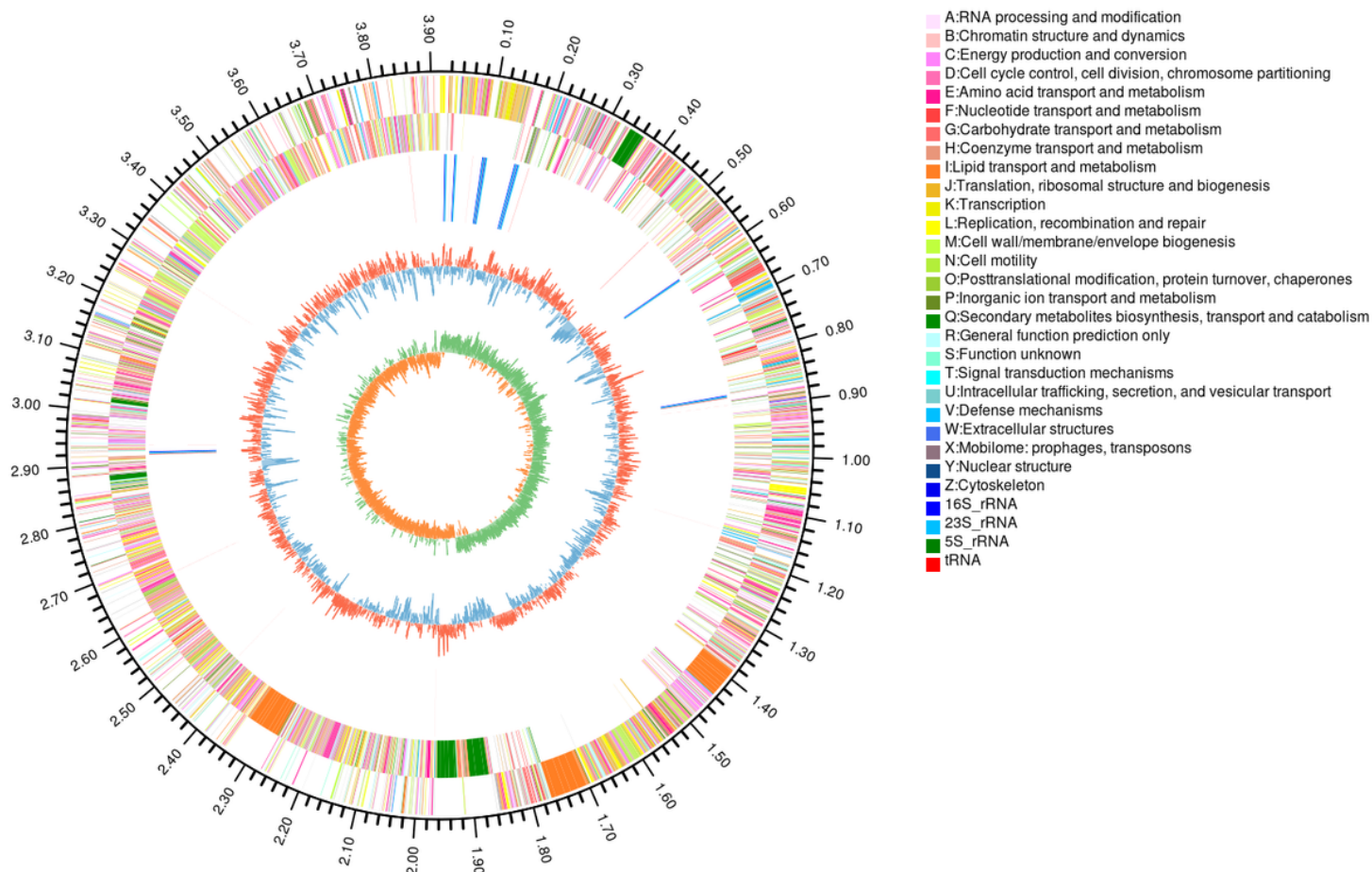


Figure 4

Circular map of *Bacillus amyloliquefaciens* GL18

Notes: The outermost circle is a marker of genome size. The second and third circles are CDSs on the positive and negative chains. Different colors indicate the functional classification of different COGs of CDSs. The fourth circle is rRNA and tRNA. The fifth circle is the GC content. The outward red part indicates that the GC content in this region is higher than the average GC content in the whole genome. The higher the peak value, the greater the difference between the GC content in this region and the average GC content. The inner circle is the GC-skew value, and the specific algorithm is $G-C/G+C$, which can assist in determining the leading and trailing lag chains. Generally, the GCskew of the leading chain is > 0 , and the GCskew of the trailing lag chain is < 0 . It can also assist in determining the starting point (minimum cumulative offset) and end point (maximum cumulative offset) of replication, especially for the circular genome.

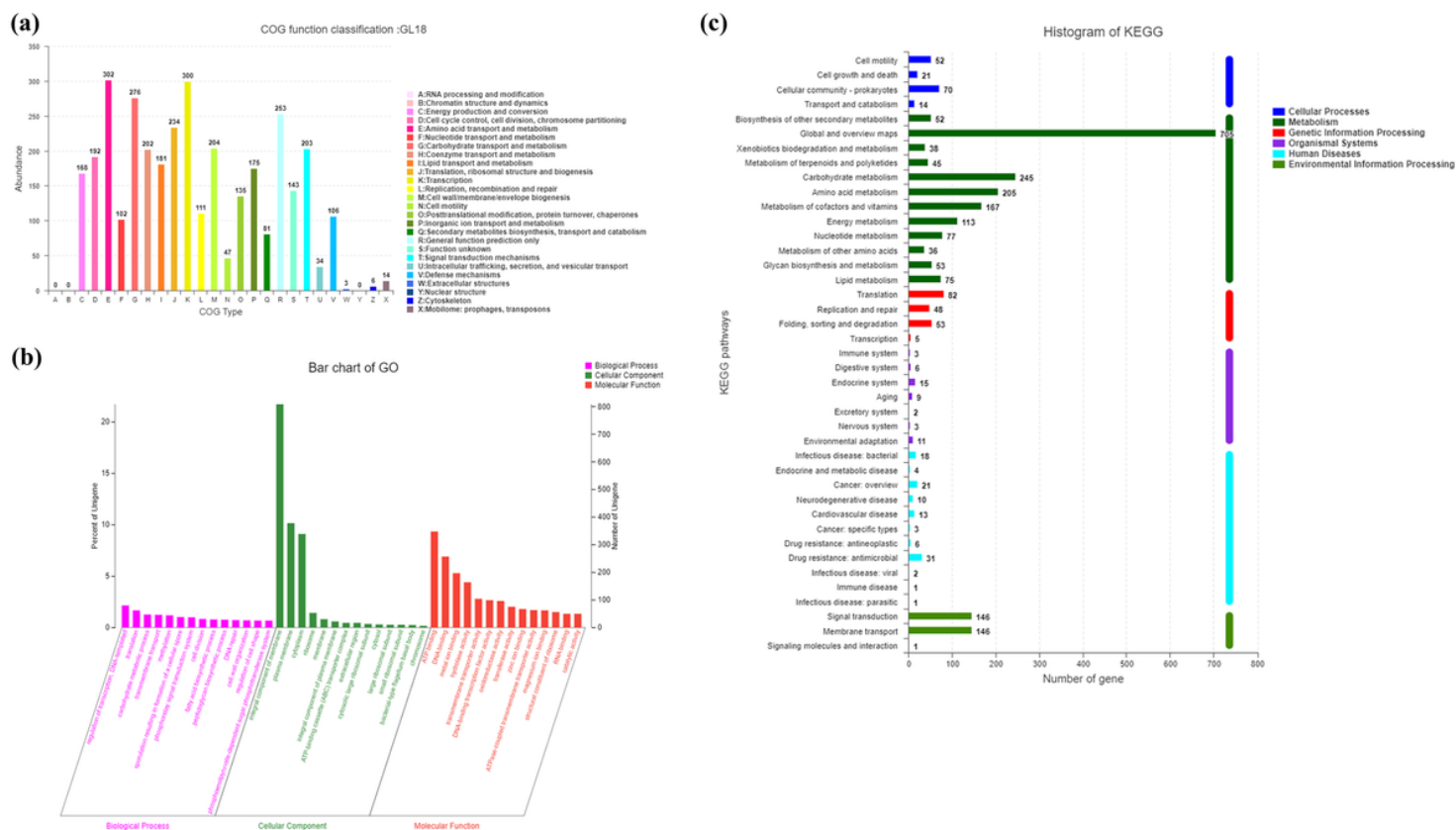
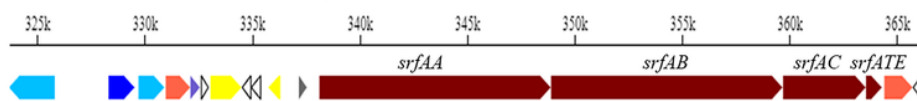


Figure 5

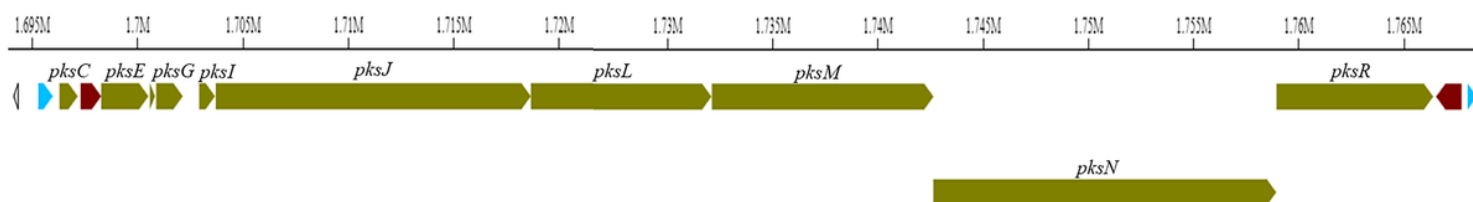
Gene function annotation of GL18.

- (a) Gene distribution based on the COG classification. (b) Gene distribution based on GO classification. (c) Classification statistics of the KEGG annotation.

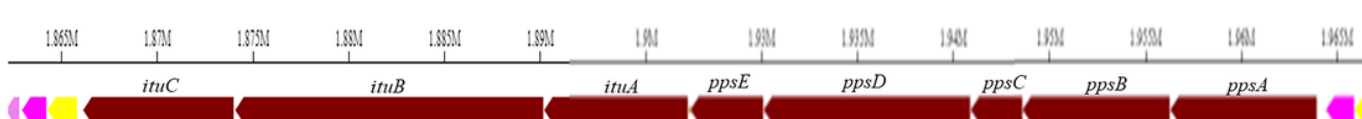
1. NRPS, Surfactin (BGC0000433)



6. TransAT-PKS, Bacillaene (BGC0001089)



7. NRPS, Fengycin (BGC0001095)



12. NRPS, Bacillibactin, (BGC0000309)

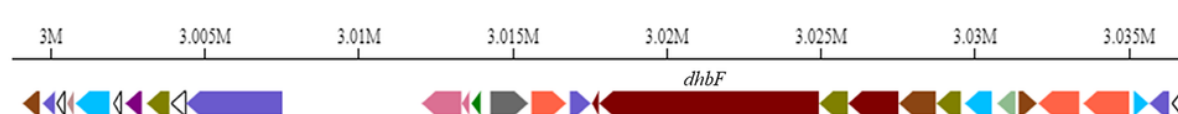


Figure 6

Biosynthetic gene clusters involved in the antagonistic activity of GL18