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Whole genome-sequence analysis of *Bacillus subtilis* strain KC14-1 with broad-spectrum antifungal activity

Xiaowei Li[1†], Yahan Chen[1*†], Shunyi Yang[1], Yi Zhou[1] and Chengde Yang[1]

Abstract

Background *Bacillus* is used as a biological control agent in agricultural production. The main mechanisms responsible for its biocontrol activity encompass the generation of various antifungal active substances during life activities, competition, antagonism with pathogens, promotion of growth, and induction of plant resistance, enhancing the inhibition of pathogenic fungi. *Bacillus* has high biological control potential and has become a research hotspot.

Results It was found that strain KC14-1 had significant inhibitory effects on *Fusarium fujikuroi*, *Rhizoclonia solani*, *Alternaria solani*, *Fusarium oxysporum*, and *Valsa mali*. Based on morphological observations, physiological and biochemical determinations, and 16 S rRNA, *gyrA*, and *gyrB* gene sequencing, strain KC14-1 was identified as *Bacillus subtilis*. Whole genome sequencing results showed that the genome of strain KC14-1 was composed of a ring chromosome 3,908,079 bp in size, with a GC content of 43.82% and 3,895 coding genes. Anti-SMASH predicted that the genome of strain KC14-1 contained nine gene clusters that synthesised antibacterial substances. The homology between fengycin, bacillibactin, pulcherriminic acid, subtilosin A, and bacilysin was 100%.

Conclusion The biocontrol potential of *Bacillus subtilis* KC14-1 was determined through whole-genome analysis. Our study provides a solid foundation for developing and utilising this strain.

Keywords Whole genome sequence, *Bacillus subtilis*, Secondary metabolites, KC14-1

Background

Plant disease incidence significantly limits agricultural production. The occurrence of plant diseases leads to an annual loss of approximately 10–15% of the global crop output, which translates into an economic loss of hundreds of billions of dollars [1] Chemical fungicides remain the primary tool used to prevent and control plant diseases [2]. However, due to their long-term use, many diseases have developed resistance [3] and severely affected the environment, threatening food safety and causing numerous other problems. Therefore, many chemical fungicides have been gradually banned, resulting in the inability to control plant diseases effectively. In particular, consumer requirements regarding the quality and safety of agricultural products have changed significantly over the past few decades, and people now attach more importance to whether agricultural products are green and healthy [4, 5], which has also led to the use of traditional pesticides to combat diseases. Therefore,

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controlling plant diseases does not rely so heavily on chemical pesticides; instead, biological pest control, rather than chemical pesticide use, is an effective measure for combating plant diseases [2, 6, 7].

Biological control refers to using microorganisms and their metabolites to inhibit the spread of pathogens [8–12]. The main bacteria used to control plant diseases are *Bacillus*. Owing to their broad-spectrum antibacterial activity and biosafety, members of the genus *Bacillus* are used as biological control agents in agricultural production [13, 14]. The main mechanisms responsible for the biocontrol activity include the production of a variety of antibacterial active substances during life activities, such as lipopeptide antibiotics (e.g., iturin [15], surfactin [16], and fengycin [17]) and antibacterial proteins (e.g., cellulase and chitinase) [18]. Competition and antagonism with pathogens can promote growth and induce resistance in plants [19, 20], thereby enhancing pathogen inhibition. Hence, competition and antagonism have a high potential for biocontrol and have become a research hotspot.

Bacillus is capable of producing a variety of secondary metabolites [21–24]. However, traditional techniques cannot efficiently and readily determine whether antagonistic strains can produce bacteriostatic substances. In addition, some potentially active antibacterial substances that have not yet been discovered are difficult to identify using traditional methods. However, with the development of biological information, whole-genome sequencing of bacteria has become the method of choice for its greater convenience and speed. Thus, through whole-genome sequencing analysis, bacteriostatic synthetic gene clusters [25] can be mined, significantly changing the situation. Indeed, to date, many researchers have analysed active secondary metabolites produced by *Bacillus* through genome sequencing [26–28]. In particular, studying functional antimicrobial genes and secondary metabolite gene clusters at the molecular level is important for mining microbial resources with the potential for biocontrol. Although many strains of secondary metabolite genes have been predicted to be resolved because of the large number of microorganisms, DNA content may significantly differ even among strains of the same species. Genomic analysis of strains with antagonistic activity is significant in supplementing the capacity to produce secondary metabolites [29, 30].

This study obtained *Bacillus* strain KC14-1, which has broad-spectrum antimicrobial activity, by screening. The classification status of this bacterium was determined based on morphological observations, physiological and biochemical analyses, and molecular biology techniques. Whole-genome component analysis, functional annotation, and analysis were performed to provide a genetic basis for further development of this bacterial strain.

Materials and methods

Antagonistic screening of biocontrol strains

Early-stage biocontrol strains KC14-1 and KC14-2 were isolated from Baiyin of Gansu Province a soil sample found in the soil interroot of unincorporated apple trees in the laboratory. *Fusarium fujikuroi*, *Rhizoclonia solani*, *Alternaria solani*, *Fusarium Oxysporum*, and *Valsa mali* were used as target fungi to evaluate the antagonistic effect of these biocontrol strains. Biological control strains and pathogenic fungi were stored at -20 °C in the College of Plant Protection, Gansu Agricultural University. Face-off culture was performed on potato agar medium, and the edges of the activated pathogenic fungi were formed into a 5-mm-thick fungal cake and inserted into the centre of the PDA medium. Biocontrol strains were inoculated on both sides of the pathogen at equal distances, and non-inoculated biocontrol strains were used as controls. Each treatment was triplicated, the colony diameter was measured at 25 °C for 5 d, and the antifungal rate was calculated. High-activity biocontrol strains were screened, and broad-spectrum activity was determined using the abovementioned method. Biocontrol bacteria were activated with LB medium, and pathogenic fungi were activated with PDA medium.

Classification and status of the antagonistic strains

GEN-III microtitre plates were used to determine the phenotypes of the biocontrol bacterial strains. This included 71 carbon-source utilisation tests and 23 chemical sensitivity tests. The strains were inoculated into LB medium and cultured at 37 °C for 12 h. The morphology of the strains was observed using field emission scanning electron microscope (FEI Co. LTD, Hillsboro, OR, USA).A bacterial genome extraction kit was used to extract bacterial genomic DNA using bacterial universal primers, 27 F (5′-AGTTTGATCMTGGCTCAG-3′) and R1492 (5′-GGTTACCTTGTTACGACTT-3′). Primer sequences p-*gyrA*-f (5′-CAGTCAGGAAATGCGTACG TCCTT-3′) and p-*gyrA*-r (5′-CAAGGTAATGCTCCAG GCATTGCT-3′) [31] were used for *gyrA* gene amplification via PCR. Similarly, PCR amplification of the *gyrB* gene was performed using primers up-1s (5′-GAAGTCA TCATGACCGTTCTGCA-3′) and up-2 sr (5′-AGCAGG GTACGGATGTGCGAGCC-3′) [32].

The PCR reaction mixture included 9.5 μL ddH_2O, upstream and downstream primers (10 μmoL/L) 1 μL, DNA template 1 μL, and 2× Rapid Taq Master Mix 12.5 μL. The PCR reaction procedure was as follows: 95 °C for 3 min; 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s, 30 cycles.

The PCR products were sent to Xi'an Qingke Biological Company(Xi'an, China) for sequencing. The sequencing results were BLAST-compared using National Centre for Biotechnology Information (NCBI) to filter out the

sequences of highly similar strains. A phylogenetic tree was constructed using MEGA 5.0 software.

Strain whole-genome sequencing

The biocontrol bacterial strains were inoculated into an LB liquid medium at 28 °C, shaken at 180 rpm, and incubated for 24 h. The fermentation broth was centrifuged at 4 °C at 10,000 rpm for 5 min to collect the bacterial pellet. DNA was extracted and commissioned to perform whole-genome sequencing by Baseo using PacBio and Illumina. The quality of the extracted DNA was examined using Qubit (Thermo Fisher Scientific, Waltham, MA, USA) and Nanodrop (Thermo Fisher Scientific).

Genome assembly and correction

The sequencing results were assembled using Falcon software (version 0.3.0) [33] and filtered using the Illumina FASTP platform (version 0.20.0) [34]. Filtering conditions: (1) removal of reads with ≥10% unrecognised nucleotides (N); (2) removal of ≥50% of reads with bases with Phred quality score≤20; (3) removal of reads targeting the barcode adapters. After filtering, the genome sequence was corrected, and the final genome sequence was determined using Pilon (version 1.23) software [35].

Prediction of genome components

Prediction of sequenced ORF genomes was performed using the NCBI database; non-coding RNA prediction was performed using rRNAmmer (version 1.2); gene island prediction was performed using IslandPath-DIMOB (version 1.0.0) in the online tool IslandViewer4; genomic CRISPR prediction was performed using the CRISPRFinder (version 4.2.17); transposon prediction was performed using TransposonPSI (version: 20100822). RepeatMasker (version 4.0.5) and TRF (version 4.09) were used to predict genomic and tandem repeats, respectively. Finally, Phage_Finder (version 2.0) was used to predict prophages [36].

Feature notes

A genosphere map of strain KC14-1 was created using CGView v2.0. Genes were annotated at NCBI by comparing non-redundant protein databases (Nr), high-quality protein annotation information databases such as SwissProt, Kyoto Encyclopaedia of Genes and Genomes (KEGG), and Gene Ontology (GO). Additionally, the Homologous Protein Cluster Database (COG) and Carbohydrate-Active EnZymes (CAZy) were used to complement the note database. Lastly, anti-SMASH (version 4.1.0) was used to predict the gene clusters of secondary metabolites [26, 37, 38].

Results

Screening of antagonistic bacterial strains

Five pathogenic fungi, namely, *F. fujikuroi*, *R. solani*, *A. solani*, *F. oxysporum*, and *V. mali*, were selected in the laboratory to test the antifungal activity of the biological control *Bacillus* strains KC14-1 and KC14-2 and the growth diameter of the pathogens on the third and seventh days, respectively. The results showed that the two bacterial strains had antifungal activity against the five fungal pathogens above; furthermore, the antifungal effect of strain KC14-1 was greater than strain KC14-2 on the third and seventh days. On the third day, strain KC14-1 showed high antagonistic activity against *Valsa mali*, with an inhibition rate of 65.48%. Later, on the seventh day, the inhibition rate of KC14-1 against *Valsa mali* reached 70%, and it had an antagonistic effect on the other four pathogens (Fig. 1; Table 1). Therefore, we believe that strain KC14-1 has broad-spectrum antifungal activity.

Identification of strain KC14-1

Strain KC14-1 was cultured in darkness at 28 °C for 24 h. The colony edges were milky white, rough, opaque, irregularly wavy, and light orange in the centre. There were noticeable bumps and folds and gram-negative staining. Scanning electron microscopy revealed that the bacteria were blunt, short, rod-like, and isolated at both ends (Fig. 2).

The phenotypic fingerprint of the strain was identified through physiological and biochemical tests using GEN-III microplates of a biological, microbial automatic-identification system. The colour changes during the REDOX reaction of tetrazolium dye indicated using carbon sources and sensitivity to chemical substances. The results showed that strain KC14-1 could use D-trehalose, sucrose, D-fructose, gentiobiose, D-cellobiose, and other carbon sources. Chemical susceptibility tests showed that strain KC14-1 grew normally in 8% NaCl (Table 2). Based on the Identification Manual of Common Bacteria, the strain was tentatively named *Bacillus* sp.

The 16 S rRNA, gyrA, and gyrB of strain KC14-1 were sequenced. The effective sequence lengths for PCR amplification were 1493, 939, and 1200 bp, respectively. The electrophoretic results for the amplified products are shown in Fig. 3. Homologous BLAST sequences were compared with those in the NCBI database. MEGA5.0 software was used to construct the phylogenetic tree of strain KC14-1 using the neighbour-joining tree method. The results showed that the 16 S rRNA sequence of KC14-1 correlated with *Bacillus subtilis* NFAA (MT192659.1) and *Bacillus subtilis* LSRBMoFPIKRGCFTRI33 (MT133340.1), clustered in the same branch. In turn, the *gyrA* gene sequence from KC14-1 and *Bacillus subtilis* SRCM100761 (CP021889.1) and

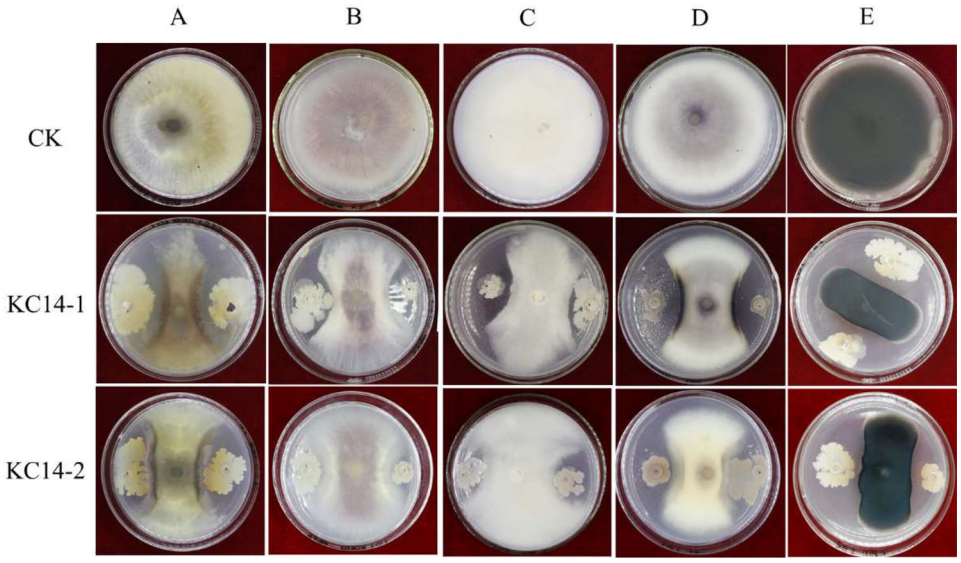

Fig. 1 Broad-spectrum activity determination of the biocontrol *Bacillus* strains KC14-1 and KC14-2. Notes: *V. mali*, **A**; *F. oxysporum*, **B**; *R. solani*, **C**; *F. fujikuroi*, **D**; *A. solani*, **E**

the *gyrB* gene sequence from *Bacillus subtilis* BEST3145 (AP024628.1) clustered in the same branch. The phylogenetic tree constructed from the amplified gene sequences was identified as *Bacillus subtilis*(Fig. 4). Based on morphological observations and physiological and biochemical characteristics, 16 S rRNA, *gyrA*, and *gyrB* were identified as genes of *Bacillus subtilis.*

Whole genome-sequence analysis of *Bacillus* strain KC14-1

Second-generation Illumina sequencing technology, third-generation PacBio sequencing technology, third-generation sequencing data for genome assembly, and second-generation data were used to correct assembly results for the *Bacillus* KC14-1 strain used in the experiments described herein.

Using the NCBI database, and after splice assembly and correction of the three-generation sequencing reads with Falcon, the genome of strain KC14-1 was found to consist of a ring chromosome with a genome size of 3,908,079 bp, GC content of 43.82%, and 3895 coding genes. Total gene length was 33,544,750 bp, the longest gene was 10,764 bp, and the shortest was 78 bp, with a GC content of 44.56% (Fig. 5). The number of tRNA genes predicted by non-coding genes was 86, with an average length of 77 bp and a total length of 6646 bp. The number of rRNA genes was 30, including 5S_rRNA, 23S_rRNA, and 16S_rRNA genes, which were 10, with lengths of 1160, 29,277, and 15,500, respectively. There were 23 sRNA genes with a total length of 3050. Using the CRISPRFinder software, CRISPR projections for the genome predicted the number of genes to be 3. The CRISPR_ Lengths were 105, 107, and 112, and the predicted Repeat_ Lengths were 25, 24, and 32, respectively.

The RepeatMasker software was used to predict the scattered repeat sequences in the bacterial strain genome. Prediction results showed that the total number of short interspersed repeat sequences (SINEs) was 12, totalling 719 bp. Meanwhile, the total number of long interspersed repeat sequences (LINEs) was 25, totalling 1650 bp. The total number of long terminal repeat (LTR) sequences was 1, with a length of 66 bp. The total number of DNA transposons (DNA elements) was three, with a length of 168 bp. A total of 42 scattered repeats were predicted with a total length of 2812 bp, accounting for 0.07% of the entire genome, and one unclassified type: tandem repeat sequence distribution of clusters on the chromosomes, including microsatellite sequences, small satellites, and satellite DNA sequences. The TRF software was used to predict 67 tandem repeats in the bacterial genome, with a total length of 6050 bp, accounting for 0.15% of the total length of the genome. Strain KC14-1 was predicted to have five genes on the genome island with lengths of 17,396, 8056, 8067, 58,116, and 54,076 bp, respectively. The total length of the genomic islands on the genome island was 145,711 bp, with an average length of 29142.20 bp. Lastly, two prophages were predicted, with genome sizes of 34,777 and 23,834 bp, respectively. The predicted total prophage length was 58,611, and the average length was 29305.50.

Basic functional notes

The Nr, Swiss-Prot, GO, KEGG, and COG databases were used for gene annotation, and BLAST was used to predict the stem gene sequences. Functional databases were used to indicate the amino acid sequences of the encoded proteins. The total number of coding genes was

Table 1 Broad-spectrum activity determination of the biocontrol *Bacillus* strains KC14-1 and KC14-2

Pathogenic fungus	3 d					7 d				
	CK (cm)	KC14-1 (cm)	KC14-1 bacteriostatic rate (%)	KC14-2 (cm)	KC14-2 bacteriostatic rate (%)	CK (cm)	KC14-1 (cm)	KC14-1 bacteriostatic rate (%)	KC14-2 (cm)	KC14-2 bacteriostatic rate (%)
V. mali	4.43 ± 0.18a	1.83 ± 0.24c	65.48 ± 7.81a	2.87 ± 0.15b	39.84 ± 2.45a	9.00 ± 0.00a	1.90 ± 0.12c	83.53 ± 1.36a	3.05 ± 0.24b	70.00 ± 2.78a
F. fujikuroi	3.45 ± 0.13a	2.90 ± 0.10b	18.16 ± 6.24b	2.80 ± 0.15b	21.63 ± 6.83b	6.82 ± 0.17a	3.03 ± 0.07b	59.79 ± 2.04c	3.27 ± 0.09b	56.21 ± 0.23 cd
R. solani	6.57 ± 0.26a	3.40 ± 0.06b	52.01 ± 2.36a	3.87 ± 0.09b	44.28 ± 3.04a	9.00 ± 0.00a	3.60 ± 0.12c	63.53 ± 1.36bc	4.03 ± 0.03b	58.43 ± 0.39c
F. oxysporum	4.02 ± 0.02a	3.05 ± 0.03c	27.48 ± 1.13b	3.60 ± 0.08b	11.87 ± 1.76b	9.00 ± 0.00a	3.38 ± 0.02c	66.08 ± 0.20b	4.53 ± 0.09b	52.55 ± 1.04d
A. solani	3.53 ± 0.08a	2.77 ± 0.17b	25.02 ± 6.77b	2.97 ± 0.08b	18.42 ± 4.86b	7.25 ± 0.13a	2.75 ± 0.10b	66.61 ± 1.97b	2.95 ± 0.05b	63.67 ± 1.17b

Data are means ± SD. Lowercase letters in the same column indicate significant differences ($p < 0.05$)

3895. The numbers of functionally annotated genes as per NR, Swiss-Prot, COG, and KEGG were 3873, 3733, 2926, and 3878, respectively, and the number of unannotated genes was 17 (Fig. 6).

The gene sequences were translated into their corresponding amino acid sequences and compared with the NR database. A total of 3873 genes were successfully annotated using the NR database. *B. subtilis*, *Bacillus* sp. *CMAA 1185*, *Bacillus* sp. *LM 4 – 2*, *Streptococcus pneumoniae*, *Bacillus* sp. *JS*, *Paenibacillus polymyxa*, *Bacillus* sp. *YP1*, *Bacillus* sp. *SN32*, *Bacillus* sp. *MBGLi79*, *B. azotoformans*, *Bacillus* sp. *Rc4*, *Bacillus cereus*, *Bacillus* sp. *FJAT-14,266*, *Bacillus halotolerans*, *Bacillus* sp. *Ru63*, *Terrabacteria*, *B. amyloliquefaciens*, *Bacillus* sp. *SJZ110*, *and Bacillus* sp. *79 – 23* had 3583, 89, 72, 50, 12, 8, 7, 6, 6, 5, 3, 3, 3, 3, 2, 2, 2, 2, 2, and 1 genes, respectively (Fig. 7).

Microbial production of secondary metabolites occurs through several metabolic pathways. It is important to study the metabolic pathways of secondary metabolites. Gene pathway annotation can effectively link the functional information of secondary metabolites with the metabolic pathways involved in cells, helping us obtain secondary metabolites more quickly, efficiently, and accurately. KEGG is a database that systematically analyses the metabolic pathways of gene products in cells and their functions as gene products. KEGG can be used to investigate the complex biological behaviours of genes further. Gene pathway annotation can be obtained according to the KEGG annotation information, which makes it easier to understand microbial function from the biological process of the system. DIAMOND was compared with the KEGG database to obtain annotation results corresponding to the genes. According to KEGG pathways, a total of 3878 genes were found to be enriched in 133 metabolic pathways, including mainly metabolism, genetic information processing, environmental information processing, cellular processes, and organic systems. Amino acid metabolism, 206 genes; carbohydrate metabolism, 267 genes; energy metabolism, 119 genes; metabolism of cofactors and vitamins, 157 genes; lipid metabolism, 70 genes; xenobiotic biodegradation and metabolism, 37 genes; biosynthesis of other secondary metabolites, 49 genes; nucleotide metabolism, 79 genes; metabolism of terpenoids and polyketones, 36 genes; and other ammonia genes. There were 55 genes involved in basal acid metabolism and 30 more in polysaccharide biosynthesis and metabolism (Fig. 8). Many genes regulate secondary metabolism in strain KC14-1. Therefore, the types of secondary metabolites in this strain may be abundant.

Gene ontology (GO) and annotation for strain KC14-1 included cytological components, molecular functions, and biological pathways. The annotation results revealed 25 functional branches of biological pathways with 14,113

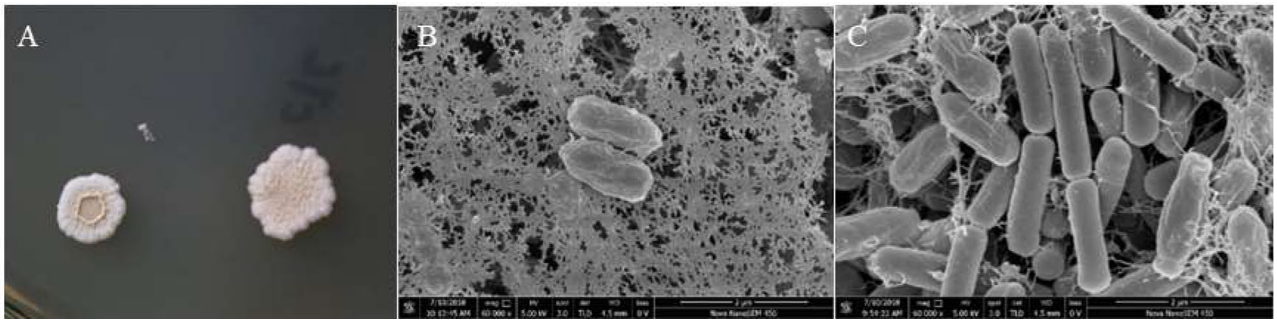

Fig. 2 Colony morphology and electron microscope morphology of Bacillus strain KC14-1. Note: **A**: Colony morphology on LB medium; **B** and **C**: Morphology of bacteria under electron microscope

Table 2 Physiological and biochemical determination of the biocontrol strain KC14-1

Carbon source utilization testing	Test result	Carbon source utilization testing	Test result	Carbon source utilization testing	Test result	Chemical sensitivity test	Test result
Negative Control	-	L-Fucose	\	D-Gluconic Acid	+	Positive Control	+
Dextrin	+	L-Rhamnose	\	D-Glucuronic Acid	-	pH 6	+
D-Maltose	-	Inosine	-	Glucuronamide	\	pH 5	-
D-Trehalose	+	D-Sorbitol	-	Mucic Acid	-	1% NaCl	+
D-Cellobiose	+	D-Mannitol	+	Quinic Acid	\	4% NaCl	+
Gentiobiose	+	D-Arabitol	-	D-Saccharic Acid	+	8% NaCl	+
Sucrose	+	myo-Inositol	\	p-Hydroxy- Phenylacetic Acid	-	1% Sodium Lactate	+
D-Turanose	-	Glycerol	+	Methyl Pyruvate	+	Fusidic Acid	\
Stachyose	-	D-Glucose-6-PO4	\	D-Lactic Acid Methyl Ester	-	D-Serine	\
D-Raffinose	+	D-Fructose-6-PO4	+	L-Lactic Acid	+	Troleandomycin	\
α-D-Lactose	-	D-Aspartic Acid	+	Citric Acid	+	Rifamycin SV	-
D-Melibiose	-	D-Serine	-	α-Keto-Glutaric Acid	-	Minocycline	\
β-Methyl-D-Glucoside	+	Gelatine	+	D-Malic Acid	-	Lincomycin	\
D-Salicin	-	Glycyl-L-Proline	-	L-Malic Acid	+	Guanidine HCl	-
N-Acetyl-D-Glucosamine	+	L-Alanine	+	Bromo-Succinic Acid	+	Niaproof 4	\
N-Acetyl-β-D-Mannosamine	+	L-Arginine	+	Tween 40	-	Vancomycin	\
N-Acetyl-D-Galactosamine	\	L-Aspartic Acid	+	γ-Amino-Butyric Acid	-	Tetrazolium Violet	\
N-Acetyl Neuraminic Acid	-	L-Glutamic Acid	+	α-Hydroxy-Butyric Acid	\	Tetrazolium Blue	\
α-D-Glucose	+	L-Histidine	+	β-Hydroxy-D, LButyric Acid	\	Nalidixic Acid	-
D-Mannose	+	L-Pyroglutamic Acid	-	α-Keto-Butyric Acid	\	Lithium Chloride	+
D-Fructose	+	L-Serine	-	Acetoacetic Acid	+	Potassium Tellurite	+
D-Galactose	-	Pectin	+	Propionic Acid	+	Aztreonam	-
3-Methyl Glucose	\	D-Galacturonic Acid	+	Acetic Acid	+	Sodium Butyrate	+
D-Fucose	\	L-Galactonic Acid Lactone	\	Formic Acid	+	Sodium Bromate	\

Note: "+" means positive; "-" means negative. "\" means that it cannot be judged accurately and is judged as a limited value

annotated genes, 15 branches of molecular functions with 5,739 annotated genes, and 19 functional branches of cytological components with 8,706 annotated genes. The numbers of genes related to cellular and metabolic processes were 2845 and 2612, respectively. Regarding molecular function, catalytic activity and binding-related genes were the highest, at 2262 and 1890, respectively. Meanwhile, the number of cell-related genes in the cytology component was the highest (2266) (Fig. 9). The cellular environment, possible biological processes, and molecular functions of stem gene products are described to understand their biological significance.

A total of 5572 protein-coding genes were annotated in the COG database. Functional annotations were divided into 25 categories of which 22 had gene enrichment: RNA processing and modification (A), chromatin structure and kinetics (B), energy production and conversion (C), cell cycle division regulation (D), amino acid transport and metabolism (E), nucleotides transport and metabolism (F), carbohydrate transport and metabolism (G), coenzyme transport and metabolism (H), lipid transport

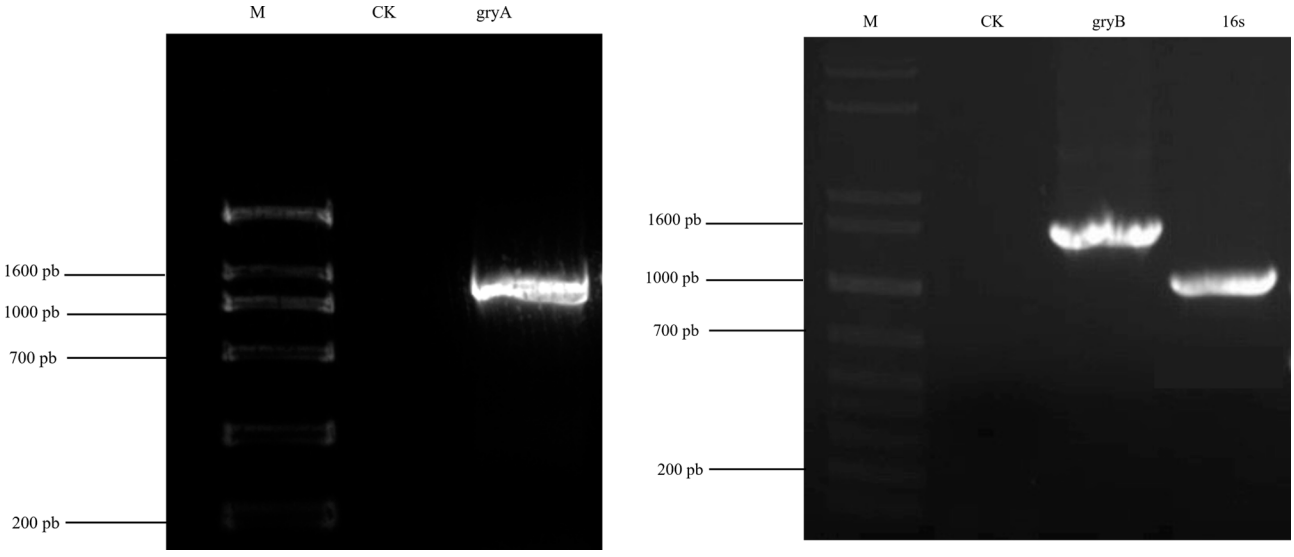

Fig. 3 PCR amplification of *gyrA*, 16 S rRNA, and *gyrB* of *B. subtilis* strain KC14-1

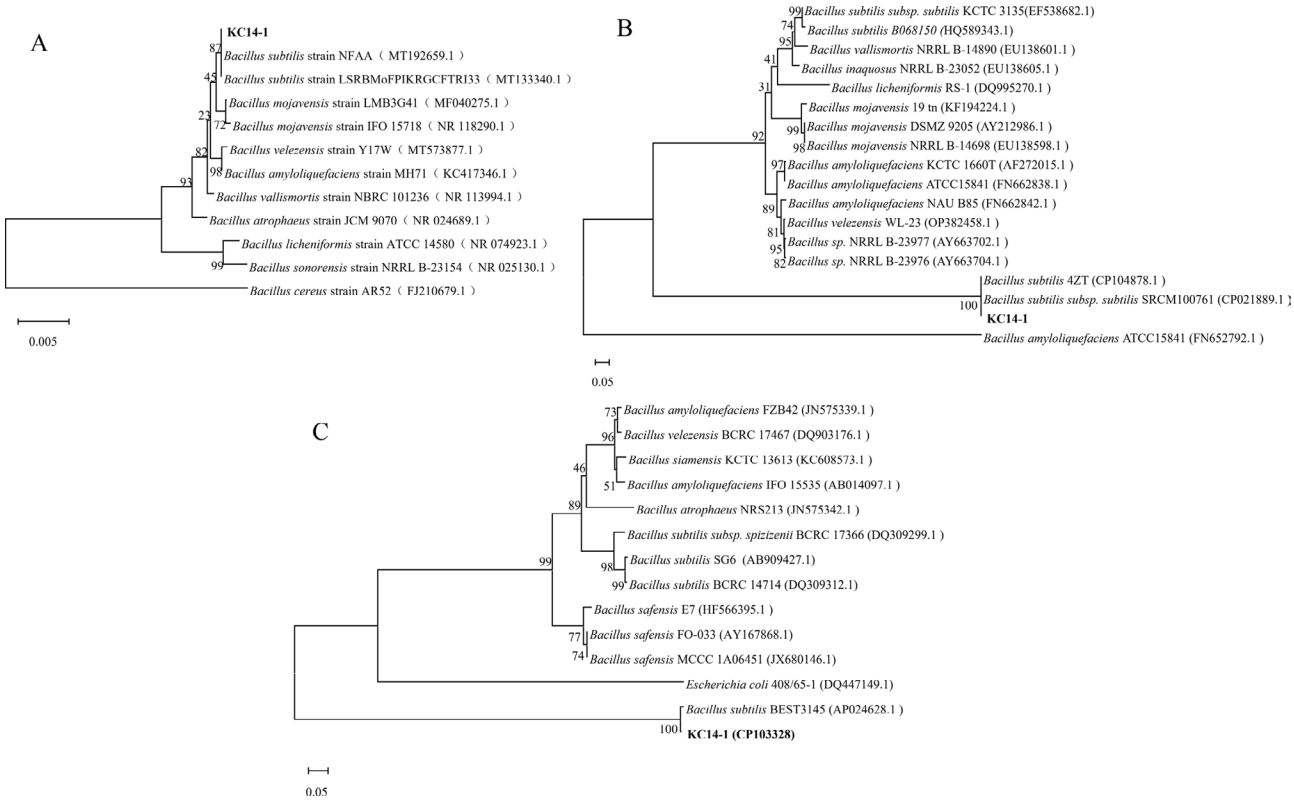

Fig. 4 Phylogenetic evolutionary tree based on (**A**) 16 S rRNA gene, (**B**) *gyrA* gene, and (**C**) *gyrB* gene

and metabolism (I), ribosome structure translation (J), transcription (K), replication (L), cell wall or membrane or extracellular envelope biosynthesis (M), cell mitosis (N), post-translational modification (O), inorganic ion transport and metabolism (P), secondary metabolite biosynthesis (Q), general function prediction of genes (R), Function unknown (S), information transduction mechanisms (T), secretion and vesicle transport (U), and defence mechanism (V). The number of successfully annotated genes for these 22 categories were 0, 2, 192, 34, 363, 83, 285, 132, 112, 167, 307, 119, 196, 66, 103, 219, 91, 493, 319, 170, 51, and 61, respectively. Additionally, there was no gene enrichment in the following three functional pathways: extracellular structure (W), nuclear structure

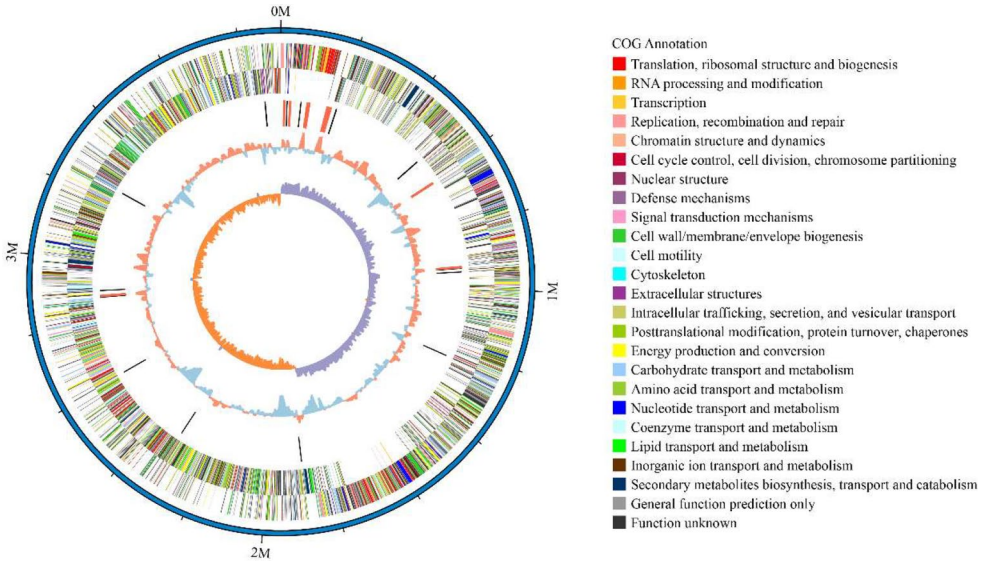

Fig. 5 Whole genome circle map of *Bacillus subtilis* KC14-1. Note: The outermost circle represents the position coordinates of the genome sequence. The figure shows the positive chain genes from the outer to the inner circle, followed by negative chain genes, ncRNA (black for tRNA, red for rRNA), GC content (red for greater than the mean, blue for less than the mean), GC SKEW, which is used to measure the relative content of G and C, and to mark the starting and end points in circular chromosomes; GC skew = (G-C)/(G + C); purple means greater than 0, orange means less than 0)

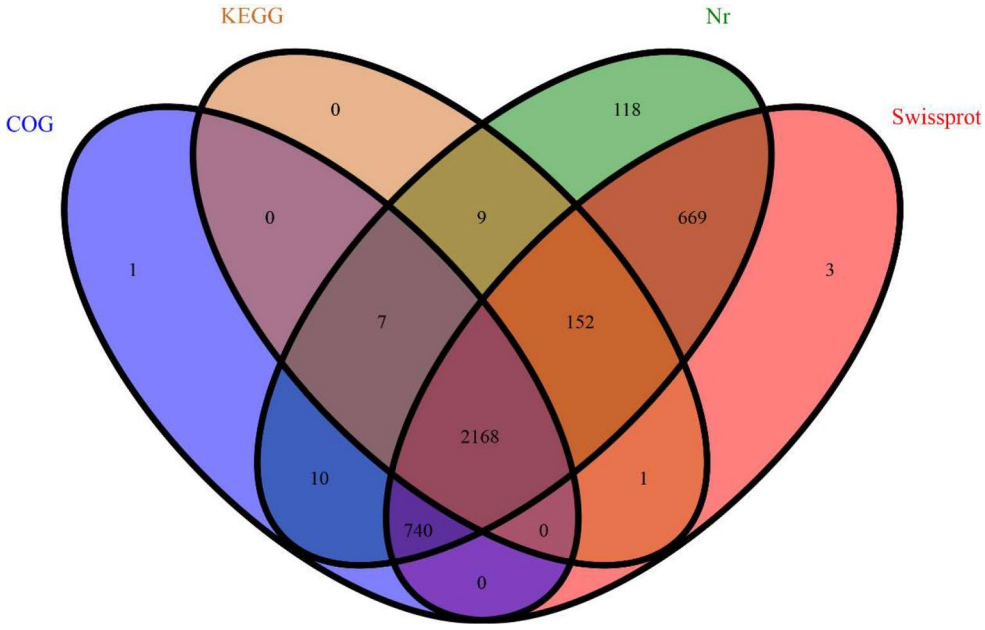

Fig. 6 Venn diagram for Nr-, Swiss-Prot-, KEGG- and COG-annotated genes

(Y), or cytoskeleton (Z) (Fig. 10). Further studies are needed to annotate genes with unknown functions.

Our results showed that six functions were identified: glycoside hydrolase (GH), glycosyltransferase (GT), polysaccharide lyase (PLs), carbohydrate esterase (CEs), auxiliary REDOX enzyme (AAs), and carbohydrate-binding modules (CBMs). The numbers of annotation genes were 216, 243, 8, 47, 2, and 99, respectively. Glycoside hydrolase (GH) and glycosyltransferase (GT) had the highest proportions among gene families. Analysis of these genes showed that the KC-14 genome contained genes encoding glucanases GH16 and CBM3, xylan-degrading enzymes GH30_8 and GH11, and peptidoglycan-degrading enzymes CBM50, GT28, CE4, and GT2, among others (Fig. 11). The gene encoding the lysozyme protein, GH23, showed that the strain could degrade dextran, xylan, and other substances.

Natural metabolite gene clusters are predicted on the anti-SMASH website. In this case, we found nine gene clusters in the genome of strain KC14-1 (Table 3).

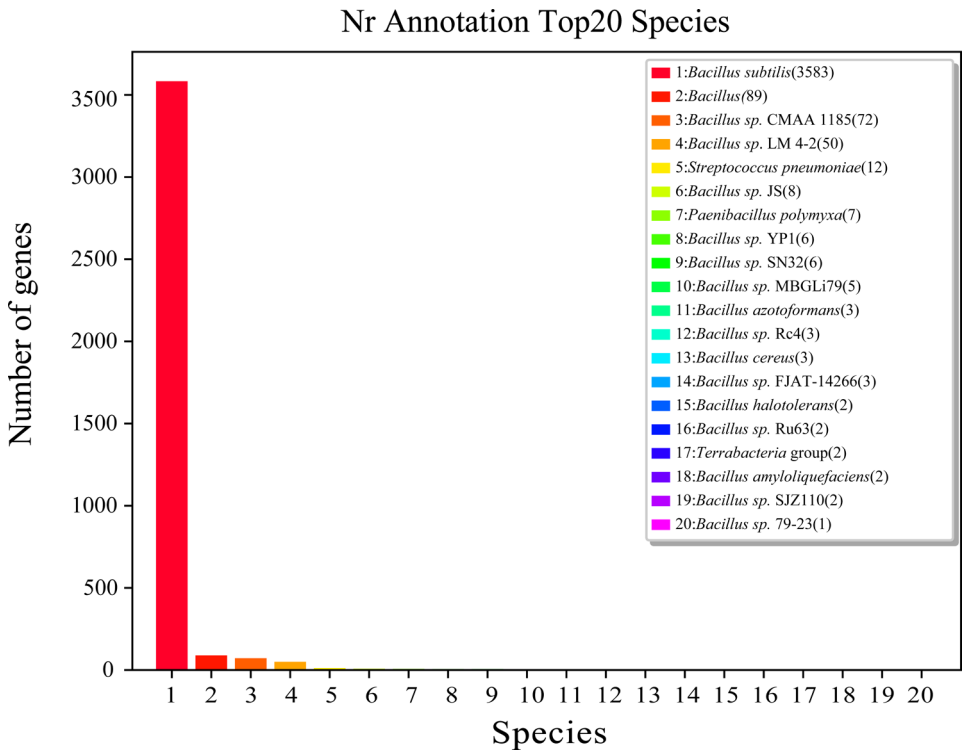

Fig. 7 Nr-annotated genes in species of *Bacillus subtilis* KC14-1 strain

Secondary metabolites are mainly produced via two metabolic pathways: non-ribosomal peptide synthase (NRPS) and polyketide synthase (PKS). In turn, the gene cluster encoding the secondary metabolism of polyketide synthases is divided into three types: type I polyketide synthases (T1pks), type II polyketide synthases (T2pks), and type III polyketide synthases (T3pks). The KC14-1 genome contains four encoded PKS and NRPS gene clusters: one PKS synthase gene cluster has two gene clusters involved in terpene synthesis, a gene cluster associated with iron vector synthesis, and a gene cluster involved in active peptide synthesis. There are five gene clusters with a similarity > 90%, which can be considered to produce this secondary metabolic gene cluster, although there may be some deviations in the predicted results on the anti-SMASH website; however, the above data still show that strain KC14-1 has a huge potential to synthesise and produce a variety of secondary metabolites.

Discussion

Bacillus is widely present in soils, aquatic environments, and plant bodies, with a wide variety and many closely related species [39, 40], making it difficult to determine their taxonomic status by any single method. Therefore, in this study, we jointly identified strain KC14-1 as *Bacillus subtilis* by morphological, physiological, and biochemical characteristics and molecular biology techniques. According to previous studies, *Bacillus* can promote plant growth [41], induce plant disease resistance, and secrete active substances against pathogens [37]. It is widely used in agriculture. For example, *Bacillus subtilis* F62 has inhibitory effects on all fungal pathogens and can reduce mycelial growth rate and decrease *Botrytis sp.* Incidence [42]. In particular, *Bacillus amyloliquefaciens* strains Trb7 and Trb1 were identified as effective bactericidal agents, effectively inhibiting *Ralstonia pseudosolanacearum* in tomato [43]; in another case, antagonistic bacteria, JF-4 and JF-5, were effective against banana wilt by 48.3% and 40.3%, respectively [44]. The *B. subtilis* strain KC14-1 consistently showed bacteriostatic activity against various pathogenic fungi, and the bacterial inhibition rate was more than 50% at day 5 compared to the control. We preliminarily believe that strain KC14-1 has the potential for biocontrol and can be further explored as a biocontrol strain.

Analysing species information at the genomic level has become an effective means of efficiently developing and using antagonistic bacterial strains, which provides an in-depth understanding of the genetic and physiological characteristics of the strains and has great significance in unravelling the underlying mechanisms of defence and their potential for the production of secondary metabolites [41, 45–47]. Via whole genome sequencing of strain KC14-1, we assembled and corrected a gene sequence consisting of a circular chromosome with a genome size of 3,908,079 bp, a GC content of 43.82%, and a coding

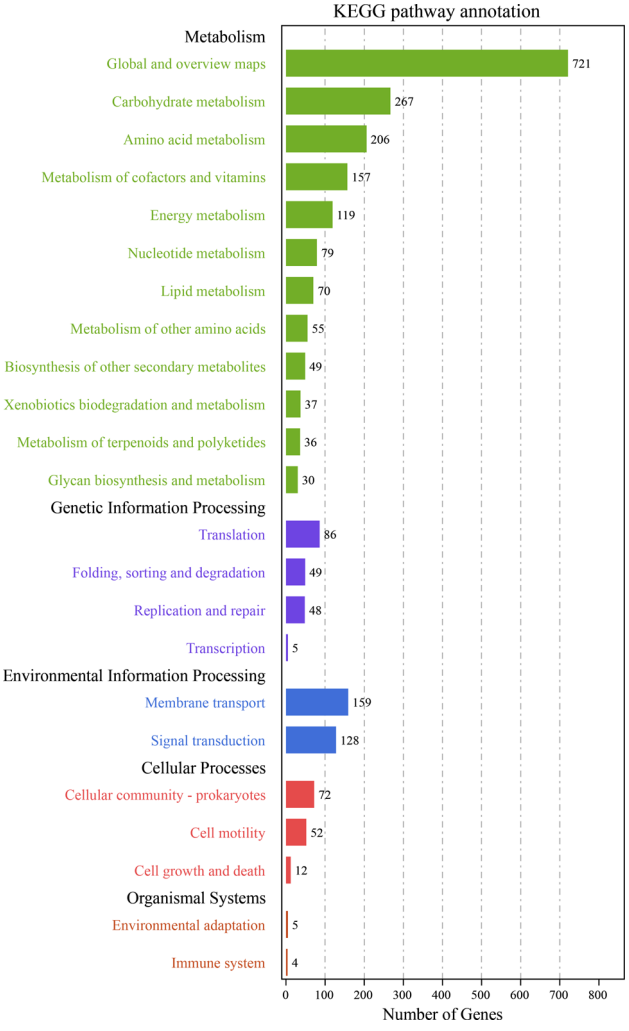

Fig. 8 KEGG-annotated genes of *Bacillus subtilis* strain KC14-1

gene number of 3895. The genome of strain KC14-1 was found to contain a variety of gene clusters involved in the synthesis of antimicrobial substances, including surfactin, 1-carbapen-2-em-3-carboxylic acid, fengycin, bacillibactin, pulcherriminic acid, subtilosin A, and bacilysin. The anti-SMASH website predictions showed high homology (100%) of the gene clusters for the synthesis of fengycin, bacillibactin, pulcherriminic acid, subtilosin A, and bacilysin, followed by 78% homology of the gene cluster for the synthesis of surfactin, and only 16% homology of the gene cluster for the synthesis of 1-carbapen-2-em-3-carboxylic acid, which does not exclude the possibility that strain KC14-1 can produce, as yet, unidentified secondary metabolites. Fengycin is produced by a variety of *Bacillus* species and inhibits pathogenic bacteria by altering the structure and permeability of pathogenic bacterial biofilms, destroying the stability and integrity of lipid membranes; it is a ferric ion chelator that chelates iron in the environment and affects the growth of pathogenic bacteria [48–50]. In turn, bacillibactin is formed by the cyclisation of glycine, threonine, and DHB and was first isolated from *B. subtilis* [51]. It is synthesised through the NRPS pathway as a high-affinity line of organisms to run iron carriers, competition, antibiosis, toxicity, and environmental remediation. It is currently believed that bacteria can inhibit pathogens owing to the ability of the ferritin to chelate iron ions in the environment, causing iron deficiency in pathogenic bacteria and nutrient deficiency leading to growth inhibition [52]. As for bacilysin, this is a dipeptide antibiotic that is active against a wide range of pathogens. Its structure is simple and has an effective mechanism of action as a pleiotropic signalling molecule, affecting cellular activity; however, not all

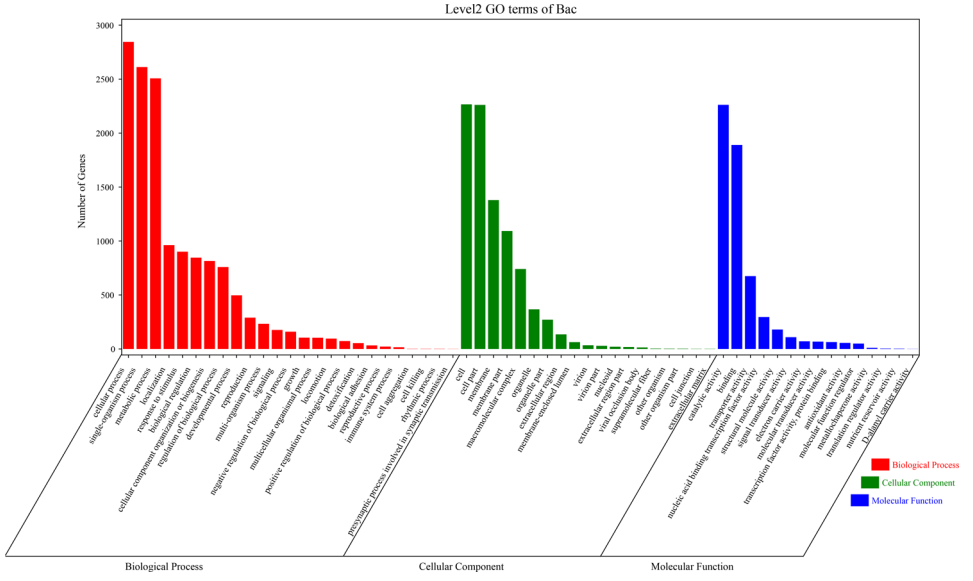

Fig. 9 GO annotation and functional classification of *Bacillus subtilis* KC14-1

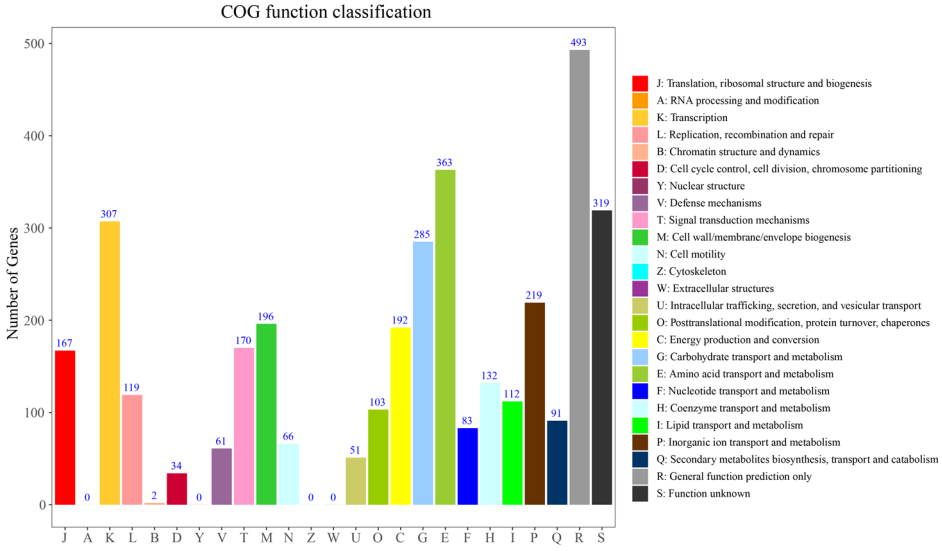

Fig. 10 COG functional classification diagram for *Bacillus subtilis* KC14-1

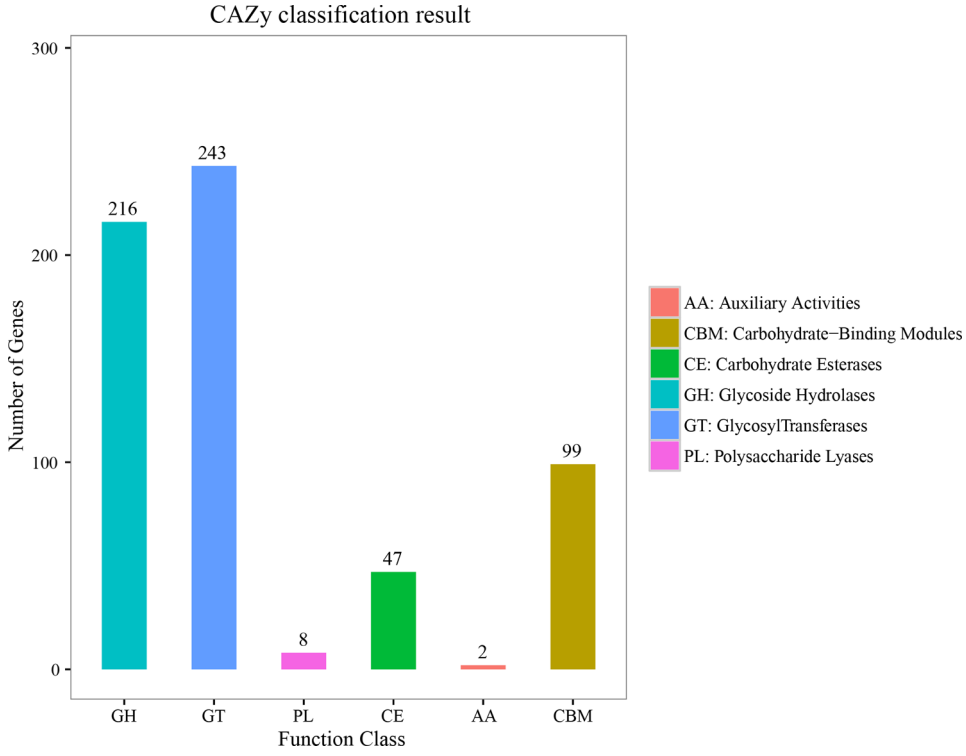

Fig. 11 CAZy classification diagram for *Bacillus subtilis* KC14-1

bacilli can produce bacilysin [53]. In turn, subtilosin A is a cyclic wool sulphur-antibiotic protein [54], a genetically encoded and ribosomally synthesised polypeptide antibiotic [55] that prevents the polymerisation of monomers in the bacterial cell wall to form functional cell wall plasmids. In addition, the KC14-1 genome contained two secondary metabolite-related gene synthesis clusters with unknown functions. The inhibitory effect of this bacterium on several pathogenic fungi demonstrated by the preliminary test may be related to known bacteriostatic active substances; however, the possibility that unknown metabolites may be involved in the inhibitory process cannot be excluded.

The genome of strain KC14-1 also revealed 615 genes encoding CAZy family members involved in the production of glucanases, xylanases, peptidoglycanases, and

Table 3 Gene clusters encoding secondary metabolites in strain KC14-1

Region	Type	From	TO	Most similar known cluster	Similarity
Region 1	NRPS, NRPS-like	354,098	419,490	surfactin	NRP: Lipopeptide
Region 2	terpene	1,124,811	1,145,608		
Region 3	NRPS, betalactone	1,845,799	1,897,348	fengycin	NRP
Region 4	terpene	1,978,609	2,000,507		
Region 5	T3PKS	2,048,239	2,089,336	1-carbapen-2-em-3-carboxylic acid	Other
Region 6	NRP-metallophore, NRPS	2,966,640	3,018,417	bacillibactin	NRP
Region 7	CDPS	3,295,582	3,316,328	pulcherriminic acid	Other
Region 8	sactipeptide	3,533,103	3,554,714	subtilosin A	RiPP: Thiopeptide
Region 9	other	3,558,022	3,599,440	bacilysin	

lysozymes. This suggests that strain KC14-1 can cause the hydrolysis of the cell wall of the pathogenic fungus, thus affecting the fungus's whole-life activity and, consequently, preventing disease damage [56–59].

Through whole genome analysis of strain KC14-1, the potential for biological control of this strain was comprehensively analysed. Secondary metabolite prediction revealed that this strain can produce a variety of antifungal active substances. Strain KC14-1 can be used for disease control by developing new fungicides and isolating its secondary active substances.

Conclusions

Bacillus subtilis strain KC14-1 showed broad-spectrum antifungal activity against various pathogenic fungi. The strain was identified as *B. subtilis* using morphological, physiological, biochemical, and molecular analyses. The whole genome of the strain was sequenced, assembled, and calibrated. The data showed that the genome of strain KC14-1 is composed of a ring chromosome with a genome size of 3,908,079 bp and a GC content of 43.82%. Analysis and functional annotation of the genomic components showed that the genome of strain KC14-1 contains various gene clusters encoding carbohydrate enzymes and secondary metabolites. The study of this bacterial genome is of great significance for the further development and utilisation of the strain and the study of the mechanisms responsible for the biological control it can exert for the benefit of agriculture.

Abbreviations

NCBI	National Center for Biotechnology Information Search database
ncRNA	non-coding RNA
KEGG	Kyoto Encyclopedia of Genes and Genomes
GO	Gene Ontology
COG	Cluster of Orthologous Groups of proteins
GIs	Genomic Islands
LTR	Long terminal repeat sequence
SINEs	short interspersed repeated sequences
LINEs	long interspersed repeated sequences
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Nr	Non-redundant protein sequence
CAZy	Carbohydrate-Active EnZymes
T1pks	type I polyketide synthases
T2pks	type II polyketide synthases
T3pks	type III polyketide synthases
NRPS	non-ribosomal peptide synthase

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Author contributions

Y.C and X.L designed this study and completed the test operation content, S. Y, Y.Z and C. Y analysed the bioinformatics of strain KC14-1,Y.C and X.L wrote the manuscript. All the authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets presented in this study can be found in the NCBI Sequence Database (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_030863625.1/) under accession no. PRJNA872326.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Peng Y, Li SJ, Yan J et al. Research Progress on Phytopathogenic Fungi and their role as Biocontrol agents [J]. Front Microbiol. 2021;12.
2. Liu R, Li J, Zhang F, et al. Biocontrol activity of *Bacillus velezensis* D4 against apple Valsa canker [J]. Biol Control. 2021;163:104760.
3. Montesinos E. Functional Peptides for Plant Disease Control [J]. Annual Review of Phytopathology, 2023, 61(Volume 61, 2023): 301 – 24.
4. Marian M, Shimizu M. Improving performance of microbial biocontrol agents against plant diseases [J]. J Gen Plant Pathol. 2019;85(5):329–36.
5. Ons L, Bylemans D, Thevissen K et al. Combining Biocontrol agents with Chemical fungicides for Integrated Plant Fungal Disease Control [J]. Microorganisms, 2020, 8(12).

6. Abdelaziz AM, Hashem AH, El-Sayyad GS, et al. Biocontrol of soil borne diseases by plant growth promoting rhizobacteria [J]. Trop Plant Pathol. 2023;48(2):105–27.
7. Chen K, Tian Z, He H, et al. *Bacillus* species as potential biocontrol agents against citrus diseases [J]. Biol Control. 2020;151:104419.
8. Weng W, Yan J, Zhou M, et al. Roles of Arbuscular mycorrhizal Fungi as a Biocontrol Agent in the control of Plant diseases [J]. Microorganisms. 2022;10(7):1266.
9. Etesami H, Jeong BR, Glick BR. Biocontrol of plant diseases by *Bacillus spp* [J]. Volume 126. Physiological and Molecular Plant Pathology; 2023. p. 102048.
10. Newitt JT, Prudence SMM, Hutchings MI, et al. Biocontrol of cereal crop diseases using *streptomycetes* [J]. Pathogens. 2019;8(2):78.
11. Bonaterra A, Badosa E, Daranas N et al. Bacteria as Biological Control Agents of Plant Diseases [J]. Microorganisms, 2022, 10(9).
12. Karačić V, Miljaković D, Marinković J, et al. *Bacillus* Speexcellentellent BiocoagentsAgents against Tdiseasesseases [J]. Microorganisms. 2024;12(3):457.
13. Xu P, Xie S, Liu W et al. Comparative Genomics Analysis provides New Strategies for Bacteriostatic ability of *Bacillus velezensis* HAB-2 [J]. Front Microbiol. 2020;11.
14. Guillén-Navarro K, López-Gutiérrez T, García-Fajardo V, et al. Broad-Spectrum Antifungal, biosurfactants and Bioemulsifier activity of *Bacillus subtilis subsp*. spizizenii—A Potential Biocontrol Bioremediat Agent Agric [J] Plants. 2023;12(6):1374.
15. Wu G, Zhou J, Zheng J, et al. Construction of lipopeptide mono-producing *Bacillus* strains and comparison of their antimicrobial activity [J]. Food Bioscience. 2023;53:102813.
16. Liu Y, Lu J, Sun J, et al. Membrane disruption and DNA binding of Fusarium Graminearum cell induced by C16-Fengycin A produced by *Bacillus amyloliquefaciens* [J]. Food Control. 2019;102:206–13.
17. Li Y, Héloir MC, Zhang X, et al. Surfactin and fengycin contribute to the protection of a *Bacillus subtilis* strain against grape downy mildew by both direct effect and defence stimulation [J]. Mol Plant Pathol. 2019;20(8):1037–50.
18. Li S, Xiao Q, Yang H, et al. Characterization of a new *Bacillus velezensis* as a powerful biocontrol agent against tomato gray mold [J]. Pestic Biochem Physiol. 2022;187:105199.
19. Hirozawa MT, Ono MA, Suguiura I M D S, et al. Lactic acid bacteria and *Bacillus spp.* as fungal biological control agents [J]. J Appl Microbiol. 2023;134(2):lxac083.
20. Aziz S, Jamshed SA, Mukhtar T, et al. Evaluation of *Bacillus spp.* as biocontrol agents against chili leaf spot caused by *Xanthomonas vesicatoria* [J]. J Plant Dis Prot. 2024;131(3):987–97.
21. Boottanun P, Potisap C, Hurdle JG, et al. Secondary metabolites from *Bacillus amyloliquefaciens* isolated from soil can kill *Burkholderia pseudomallei* [J]. AMB Express. 2017;7(1):16.
22. Fira D, Dimkić I, Berić T, et al. Biological control of plant pathogens by *Bacillus* species [J]. J Biotechnol. 2018;285:44–55.
23. Mohamad OAA, Li L, Ma J-B, et al. Evaluation of the antimicrobial activity of Endophytic Bacterial Populations from Chinese Traditional Medicinal Plant Licorice and characterization of the Bioactive secondary metabolites produced by *Bacillus atrophaeus* against *Verticillium dahliae* [J]. Frontiers in Microbiology; 2018. p. 9.
24. Sun Y, Liu W-C, Shi X, et al. Inducing secondary metabolite production of *aspergillus sydowii* through microbial co-culture with *Bacillus subtilis* [J]. Microb Cell Fact. 2021;20(1):42.
25. Ma X-H, Shen S, LI W, et al. Bioherbicidal potential of *Bacillus altitudinis* D30202 on Avena fatua L.: a whole-genome sequencing analysis [J]. J Appl Genet. 2023;64(4):809–17.
26. Shi C, Zeng S, Gao X et al. Complete Genome Sequence Analysis of *Bacillus subtilis* MC4-2 Strain That against Tobacco Black Shank Disease [J]. International Journal of Genomics, 2024, 2024: 1–19.
27. Jatan R, Kamboj R, Kumar M, et al. Isolation and whole genome sequencing of *Pseudomonas aeruginosa* strain RK1 and its biocontrol potential against phytopathogens of rice [J]. Biologia. 2023;78(9):2357–69.
28. Hafeez AB, Pełka K, Buzun K, et al. Whole-genome sequencing and antimicrobial potential of bacteria isolated from Polish honey [J]. Appl Microbiol Biotechnol. 2023;107(20):6389–406.
29. Babalola OO, Adeleke BS, Ayangbenro AS. Whole genome sequencing of sunflower Root-Associated *Bacillus cereus* [J]. Evolutionary Bioinf. 2021;17:11769343211038948.
30. Gan S, Ruan L, XU X, et al. Whole genome sequencing and analysis of *Bacillus* sp. TTMP2, a tetramethylpyrazine-producing bacterium [J]. Mol Biol Rep. 2024;51(1):863.
31. Yamamoto S, Harayama S. PCR amplification and direct sequencing of gyrB genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains [J]. Appl Environ Microbiol. 1995;61(3):1104–9.
32. Roberts MS, Nakamura LK, Cohan FM. *Bacillus mojavensis* sp. distinguishable-shable from *Bacillus subtilis* by Sisolationladivergencergence isequencequencedifferencesrencfatty acidycompositionsition [J]. Int J Syst Evol MicroBiol. 1994;44(2):256–64.
33. Chin C-S, Peluso P, Sedlazeck FJ, et al. Phased diploid genome assembly with single-molecule real-time sequencing [J]. Nat Methods. 2016;13(12):1050–4.
34. Chen S, Zhou Y, Chen Y, et al. Fastp: an ultra-fast all-in-one FASTQ preprocessor [J]. Bioinformatics. 2018;34(17):i884–90.
35. Wang J, Walker BJ, Abeel T et al. Pilon: an Integrated Tool for Comprehensive Microbial Variant Detection and Genome Assembly Improvement [J]. PLoS ONE. 2014;9(11).
36. Li A, Wang M, Zhang Y et al. Complete genome analysis of *Bacillus subtilis* derived from yaks and its probiotic characteristics [J]. Front Veterinary Sci. 2023;9.
37. Zhou Z, Tang X, Peng L, et al. Complete genome sequence of *Bacillus velezensis* GUAL210, a potential Biocontrol Agent isolated from Pepper Rhizosphere [J]. Plant Dis. 2023;107(3):915–8.
38. Xie L, Liu L, Luo Y et al. Complete genome sequence of biocontrol strain *Bacillus velezensis* YC89 and its biocontrol potential against sugarcane red rot [J]. Front Microbiol. 2023;14.
39. Lim SB, Y, Junqueira ACM, Uchida A, et al. Genome sequence of *Bacillus velezensis* SGAir0473, isolated from Tropical Air Collected in Singapore [J]. Genome Announcements. 2018;6(27). https://doi.org/10.1128/genomea.00642-18.
40. Owusu-Darko R, Allam M, Ismail A et al. Comparative Genome Analysis of *Bacillus sporothermodurans* with Its Closest Phylogenetic Neighbor, *Bacillus* oleronius, and *Bacillus cereus* and *Bacillus subtilis* Groups [J]. Microorganisms, 2020, 8(8): 1185.
41. Abdel-Moghies AH, El-Sehrawy MH, Zakaria AE, et al. In vivo application of potent probiotics for enhancing potato growth and controlling *Ralstonia solanacearum* and *Fusarium oxysporum* infections [J]. Antonie Van Leeuwenhoek. 2024;117(1):33.
42. König F, Sandri MR, Russi A, et al. Biocontrol of tomato pathogens by *Bacillus subtilis* F62 and its synergistic action in plant growth promotion with *Rhizobium sp.* L5 [J]. Biocontrol Sci Technol. 2024;34(6):551–65.
43. Balamurugan A, Ashajyothi M, Velmurugan S, et al. *Bacillus amyloliquefaciens*: a versatile antimicrfirmicutemicute for the suppression of wilt caused by *Ralstonia pseudosolanacearum* in tomato [J]. Biocontrol Sci Technol. 2024;34(4):336–54.
44. Duan Y, Pang Z, Yin S et al. Screening and analysis of antifungal strains *Bacillus subtilis* JF-4 and *B. Amylum* JF-5 for the Biological Control of *Fusarium* Wilt of Banana [J]. J Fungi. 2023;9(9).
45. Li L, Wang R, Liang X et al. Characterization of a *Bacillus velezensis* with antibacterial activity and its inhibitory effect on Gray Mold germ [J]. Agronomy. 2023;13(6).
46. Liu H, Zeng Q, Yalimaimaiti N, et al. Comprehensive genomic analysis of *Bacillus velezensis* AL7 reveals its biocontrol potential against Verticillium wilt of cotton [J]. Mol Genet Genomics. 2021;296(6):1287–98.
47. Luo Y, Chen L, Lu Z et al. Genome sequencing of biocontrol strain *Bacillus amyloliquefaciens* Bam1 and further analysis of its heavy metal resistance mechanism [J]. Bioresources Bioprocess. 2022;9(1).
48. Wang D, Zhan Y, Cai D, et al. Regulation of the synthesis and secretion of the Iron Chelator Cyclodipeptide Pulcherriminic Acid in *Bacillus licheniformis* [J]. Appl Environ Microbiol. 2018;84(13):e00262–18.
49. Wang S, Wang H, Zhang D, et al. Multistep Metabolic Engineering of *Bacillus licheniformis* to improve Pulcherriminic Acid production [J]. Appl Environ Microbiol. 2020;86(9):e03041–19.
50. Zhang H, Wang S, Deng Q, et al. The effect of pulcherriminic acid produced by Metschnikowia citriensis in controlling postharvest diseases of citrus fruits [J]. Pestic Biochem Physiol. 2023;197:105657.
51. May JJ, Wendrich TM, Marahiel MA. The dhb operon of *Bacillus subtilisEncodes* the Biosynthetic Template for the Catecholic Siderophore 2,3-Dihydroxybenzoate-Glycine-threonine trimeric Ester Bacillibactin* [J]. J Biol Chem. 2001;276(10):7209–17.
52. Yu X, Ai C, Xin L, et al. The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper [J]. Eur J Soil Biol. 2011;47(2):138–45.

53. Islam T, Rabbee MF, Choi J et al. Biosynthesis, Molecular Regulation, and Application of Bacilysin Produced by *Bacillus* Species [J]. Metabolites, 2022, 12(5): 397.
54. Algburi A, Zehm S, Netrebov V, et al. Subtilosin prevents Biofilm formation by inhibiting bacterial quorum sensing [J]. Probiotics Antimicrob Proteins. 2017;9(1):81–90.
55. Thennarasu S, Lee D-K, Poon A, et al. Membrane permeabilization, orientation, and antimicrobial mechanism of subtilosin A [J]. Chem Phys Lipids. 2005;137(1):38–51.
56. Xu T, Zhu T, Li S. β-1,3 – 1,4-glucanase gene from *Bacillus velezensis* ZJ20 exerts antifungal effect on plant pathogenic fungi [J]. World J Microbiol Biotechnol. 2016;32(2):26.
57. Qin Z, Yu S, Zhang K, et al. Characterization of a glycoside hydrolase family 157 Endo-β-1,3-Glucanase that displays antifungal activity against phytopathogens [J]. J Agric Food Chem. 2023;71(27):10383–92.
58. Brown AE. Activity of glucanases of Zygorrhynchus moelleri in relation to antagonism against some soil-borne plant pathogenic Fungi [J]. J Phytopathol. 1987;120(4):298–309.
59. Yu M, Zhang G, Jiang J, et al. Lysobacter enzymogenes employs diverse genes for inhibiting Hypha Growth and Spore germination of soybean fungal pathogens [J]. Phytopathology®. 2020;110(3):593–602.

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